

LOAN DOCUMENT

		PHOTOGRAPH THIS SHEET <div style="border: 1px solid black; width: 100px; height: 80px; margin: 0 auto;"></div>	INVENTORY <div style="border: 1px solid black; width: 100px; height: 80px; margin: 0 auto; text-align: center; font-size: 2em;">①</div>
DTIC ACCESSION NUMBER	LEVEL <div style="border: 1px solid black; width: 100px; height: 40px; margin: 0 auto;"></div>	DOCUMENT IDENTIFICATION <div style="border: 1px solid black; padding: 5px; margin: 0 auto;"> AFRL-ML-TY-TB-1998-4522 Jul 98 </div>	
	<div style="border: 1px solid black; padding: 10px; margin: 0 auto;"> DISTRIBUTION STATEMENT A Approved for public release Distribution Unlimited </div>		
	DISTRIBUTION STATEMENT		
ACCESSION FOR NTIS <input type="checkbox"/> GRAM <input checked="" type="checkbox"/> DTIC <input type="checkbox"/> TRAC <input type="checkbox"/> UNANNOUNCED <input type="checkbox"/> JUSTIFICATION		<div style="border: 1px solid black; width: 100%; height: 150px;"></div>	
BY			
DISTRIBUTION/			
AVAILABILITY CODES			
DISTRIBUTION <input type="checkbox"/> AVAILABILITY AND/OR SPECIAL <input type="checkbox"/>		<div style="border: 1px solid black; width: 100%; height: 100px;"></div>	
A-1			
DISTRIBUTION STAMP		DATE ACCESSIONED	
DTIC QUALITY INSPECTED 1		DATE RETURNED	
		REGISTERED OR CERTIFIED NUMBER	
DATE RECEIVED IN DTIC		PHOTOGRAPH THIS SHEET AND RETURN TO DTIC-FDAC	

HANDLE WITH CARE

AFRL-ML-TY-TR-1998-4522



**DRAFT TECHNICAL PROTOCOL: A TREATABILITY
TEST FOR EVALUATING THE POTENTIAL
APPLICABILITY OF THE REDUCTIVE ANAEROBIC
BIOLOGICAL IN SITU TREATMENT TECHNOLOGY
(RABITT) TO REMEDIATE CHLOROETHENES**

**JEFF J. MORSE, BRUCE C. ALLEMAN, JAMES M. GOSSETT, STEPHEN H.
ZINDER, DONNA E. FENNELL, GUY W. SEWELL, CATHERINE M. VOGEL**

BATTELLE MEMORIAL INSTITUTE

505 KING AVENUE

COLUMBUS, OHIO 43201-2693

SCHOOL OF CIVIL ENGINEERING, THE SECTION OF MICROBIOLOGY

CORNELL UNIVERSITY

ITHACA, NY 14853

JULY 1998

FINAL REPORT: APRIL 1997 – JULY 1998

Approved for public release; distribution is unlimited

**DESTRUCTION NOTICE – Destroy by any method that will prevent disclosure of contents or
reconstruction of this document.**

**AIR FORCE RESEARCH LABORATORY
MATERIALS & MANUFACTURING DIRECTORATE
AIRBASE & ENVIRONMENTAL TECHNOLOGY DIVISION
TYNDALL AFB FL 32403-5323**

NOTICES

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, not any employees, nor any of their contractors, subcontractors, or their employees, make any warranty, expressed or implied, or assume any legal liability or responsibility for the accuracy, completeness, or usefulness of any privately owned rights. Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency, contractor, or subcontractor thereof. The views and opinions of the authors expressed herein do not necessarily state or reflect those of the United States Government or any agency, contractor, or subcontractor thereof.

When Government drawings, specifications, or other data are used for any purpose other than in connection with a definitely Government-related procurement, the United States Government incurs no responsibility or any obligation whatsoever. The fact that the Government may have formulated or in any way supplied the said drawings, specifications, or other data, is not to be regarded by implication, or otherwise in any manner construed, as licensing the holder to any other person or corporation; or as conveying any rights or permission to manufacture, use, or sell any patented invention that may in any way be related thereto.

This technical report has been reviewed by the Public Affairs Office (PA) and is releasable to the National Technical Information Service (NTIS) where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

FOR THE COMMANDER:



CATHERINE M. VOGEL, P.E.
Biotechnology Program Manager



CHRISTINE WAGENER-HULME, Lt Col, USAF
Chief, Environmental Technology
Development Branch

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 1998		3. REPORT TYPE AND DATES COVERED Final Report: April 1997 - July 1998
4. TITLE AND SUBTITLE DRAFT TECHNICAL PROTOCOL - A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) to Remediate Chloroethenes			5. FUNDING NUMBERS Contract No. F08637-95-C-6004 Task 4 and Contract No. F0863797C6021	
6. AUTHOR(S) Jeff J. Morse, Bruce C. Alleman, James M. Gossett, Stephen H. Zinder, Donna E. Fennell, Guy W. Sewell, and Catherine M. Vogel				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693 and School of Civil and Environmental Engineering and the Section of Microbiology, Cornell University, Ithaca, NY 14853			8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Air Force Research Laboratory Airbase & Environmental Technology Division 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida 32403-5323			10. SPONSORING/MONITORING AGENCY REPORT NUMBER AFRL-ML-TY-TR-1998-4522	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE A	
13. ABSTRACT (Maximum 200 words) This draft, unvalidated protocol describes a comprehensive approach for conducting a phased treatability test to determine the potential for employing the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) at any specific site. The treatability test is presented in a phased approach, allowing the user to screen out RABITT in the early stages of the process to save time and cost. The first phase relies on a review of existing site data, which include any data generated during previous site investigations. The second phase requires that a candidate test plot location within the plume be selected for more detailed characterization. The decision to proceed to the third phase of the treatability study is based on the evaluation of the data collected during the characterization of the candidate test plot. The third phase of the treatability study involves conducting laboratory microcosm studies. The fourth phase entails field testing of the electron donor/nutrient formulation determined in the laboratory microcosm studies to be the most effective for supporting biologically mediated reductive dechlorination. The data from the phased treatability test described in this protocol indicate the potential for the microbiological component of RABITT and are used to make the decision to proceed to pilot-scale testing or full-scale implementation of RABITT. This unvalidated protocol was developed by a team of government, industry, and academic scientists and engineers and will be demonstrated at five DoD field sites over the next two years. The protocol will be applied during 1998 and 1999 at five field demonstration sites representing the Air Force, Army, and Navy. Selection of sites in several EPA Regions plus (cont)				
14. SUBJECT TERMS chloroethene(s), tetrachloroethene, trichloroethene, anaerobic, microcosm, treatability, biodegradation, in situ			15. NUMBER OF PAGES 100	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

14. Abstract cont:

interactions with the Interstate Technology Regulatory Cooperation (ITRC) Work Group will facilitate improved regulatory acceptance of this innovative technology. After the five field demonstrations are completed, the suggested system design, monitoring strategy, sampling and analytical methods will be assessed, revised if needed, and the protocol finalized. Publication of the finalized protocol is anticipated for early 2000.

PREFACE

This report was prepared by the Battelle Memorial Institute, 505 King Avenue, Columbus, Ohio 43201-2693, under Task 4 of Contract No. F08637-95-D-6004 and the School of Civil and Environmental Engineering and the Section of Microbiology at Cornell University, Ithaca, NY 14853 under Contract No. F0863797C6021, for the Air Force Research Laboratory Airbase & Environmental Technology Division (AFRL/MLQ), 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida 32403-5323.

This draft protocol describes a comprehensive approach for conducting a phased treatability test to determine the potential for implementing the **Reductive Anaerobic Biological In Situ Treatment Technology (RABITT)** at any specific site. It is intended to be a stand-alone document that provides guidance to site managers, contractors, and regulators. After applying this unvalidated protocol at five Department of Defense field sites, the suggested approach will be assessed and the protocol finalized.

The DoD Environmental Security Technology Certification Program (ESTCP) funded this effort.

Drafting of the RABITT protocol was performed between April 1997 and February 1998. The AFRL/MLQ project officer was Catherine M. Vogel. Erica Becvar, ARA, provided significant assistance in the management of this effort.

Executive Summary

The chloroethenes, primarily tetrachloroethene (PCE) and trichloroethene (TCE), have been widely used for a variety of industrial processes. Use, disposal practices, accidental spills, and a lack of understanding of the fate of these chemicals in the environment have led to widespread contamination at U.S. Department of Defense (DoD) and industrial facilities worldwide. Extensive research has been conducted to develop technologies for remediating both groundwater and soils at sites contaminated with this group of compounds. In situ bioremediation is a technology area that has shown promise for chlorinated ethene remediation. Of the in situ bioremediation technologies, the reductive anaerobic biological in situ treatment technology (RABITT), an enhanced anaerobic dechlorination process, is the most promising. RABITT offers the potential for destruction of PCE and less chlorinated chloroethenes by the addition of an electron donor/nutrient formulation to the subsurface. This technique can eliminate the requirement for aboveground treatment. Although RABITT may have potential for widespread application, various hydrogeologic, geochemical, and biological considerations may preclude its use at a given site.

This draft protocol describes a comprehensive approach for conducting a phased treatability test to determine the potential for employing RABITT at any specific site. It is not meant as a guide for designing either full or pilot-scale in situ biotreatment systems for chlorinated ethenes or any other contaminant. After applying this draft protocol at five independent sites, the suggested monitoring, sampling and analytical methods will be assessed and the protocol finalized.

The treatability test is presented in a phased approach, allowing the user to screen out RABITT in the early stages of the process to save time and cost. The protocol guides the user through a decision process in which information is collected and evaluated to determine if the technology should be given further consideration. RABITT will be screened out if it is determined that site-specific characteristics, regulatory constraints, or other logistic problems suggest that the technology will be difficult or impossible to employ, or if a competing technology clearly is superior.

The first phase of the approach relies on a review of existing site data, which include any data generated during previous site investigations, comprising contaminant types and concentrations; site hydrogeologic information such as stratigraphy, groundwater elevations, flow direction and velocity; and aquifer parameters such as porosity and hydraulic conductivity. Information on site usage, including chemicals used or disposed of at the site and the schedule of operation, should be included in

this phase of the decision process. Any data obtained are used to develop a conceptual model of the site. The decision to proceed with the RABITT screening process should be supported by data indicating that the site meets the requirements for successful technology application.

The second phase of the approach requires that a candidate test plot location within the plume be selected for more detailed characterization. The location is selected based on the data review conducted in the first phase. Second-phase characterization activities will examine contaminant, geochemical, and hydrogeologic parameters on a relatively small scale to determine the selected location's suitability as a RABITT test plot. Field methods will be dictated by site-specific conditions and include drilling, the collection of aquifer cores, and discrete-depth groundwater sampling. Discrete depth groundwater samples are analyzed for contaminant concentrations and a suite of geochemical parameters, including electron acceptor concentrations. The data are used to define a vertical profile of these parameters at each location. Aquifer core material is examined to delineate the stratigraphy at each location, and samples of the core are collected and submitted for contaminant, geochemical, and porosity testing.

The decision to proceed to the third phase of the treatability study is based on the evaluation of the data collected during the characterization of the candidate test plot. In instances where unexpected unfavorable results are uncovered, the RABITT process should be discontinued until the differences between the initial site characterization data used to select the candidate location and the data collected at that location can be reconciled. If further investigation reveals that the site does not meet RABITT criteria, the technology is dropped from consideration. Only sites meeting the screening criteria proceed to the third phase.

The third phase of the treatability study involves conducting laboratory microcosm studies. These studies are conducted to determine if RABITT has potential for application at a site, and if it does, to determine what electron donor/nutrient formulation should be field-tested to provide optimum biological degradation performance. The microcosms are set up using a number of electron donor and nutrient combinations and are monitored over time to evaluate each amendment for its effectiveness in supporting chloroethene dechlorination. Microcosms will be incubated for six months or 3 PCE/TCE depletion cycles, whichever comes first. The formulation that supports the most rapid and complete dechlorination of chloroethenes is recommended for field testing in the final phase of the screening process. If the results from the microcosm tests indicate that reductive dechlorination does not occur in response to the addition of the electron donor/nutrient formulation, the technology is eliminated from further consideration.

The fourth and final phase of the treatability test entails field testing the electron donor/nutrient formulation determined in the laboratory microcosm to be the most effective for supporting biologically mediated reductive dechlorination. A field-scale test system and a testing approach are described. The system described in the protocol consists of three injection wells, two extraction wells, and a series of nested monitoring wells located between the injection and extraction wells. The three closely spaced injection wells inject contaminated site groundwater that has been extracted from a downgradient extraction well and amended with electron donor and nutrients. The simultaneous injection and extraction of site groundwater at opposite ends of the test plot impose a hydraulic gradient that directs local groundwater flow. Because this system requires the extraction, injection and possibly the discharge of contaminated groundwater, regulatory approval must be secured before initiating any field activities.

RABITT performance is assessed by collecting samples from the monitoring locations and analyzing them for contaminant type and level, dechlorination products, electron donor and electron-donor degradation products, and other relevant geochemical parameters. Although it is desirable to achieve complete dechlorination, a demonstration in which PCE and/or TCE are effectively dechlorinated to dichloroethene (DCE) and/or vinyl chloride (VC) may not necessarily be considered a failure. The protocol encourages criteria for success to be user defined and based on site-specific project goals. For the purposes of evaluating the protocol, the first five sites tested will be considered successful if a cost-effective acceleration of chloroethene remediation is observed. Based on this criterion, tests demonstrating an enhanced rate of transformation from PCE to DCE could be considered successful if the overall rate of complete dechlorination to ethene by natural attenuation is accelerated.

The data from the phased treatability test described in this protocol indicate the potential for the microbiological component of RABITT and are used to make the decision to proceed to pilot-scale testing or full-scale implementation of RABITT. For small plumes that can be treated without the need for groundwater manipulation, pilot-scale testing may not be necessary. For sites with large plumes that could require considerable groundwater manipulation, a pilot test is recommended. The focus of pilot testing is to obtain the data necessary to effectively design a full-scale system, which requires a detailed analysis of the hydraulic properties of the aquifer within the plume. At sites already undergoing treatment, RABITT may be coupled effectively to the existing technology to enhance treatment performance and reduce the time required to achieve the treatment goal.

Table of Contents

EXECUTIVE SUMMARY	i
LIST OF FIGURES	vii
LIST OF TABLES	vii
LIST OF ABBREVIATIONS	viii
1.0 PROTOCOL OVERVIEW	1
1.1 Protocol Objective	1
1.2 Protocol Scope	1
1.3 Protocol Layout	2
2.0 INTRODUCTION	4
2.1 In Situ Biodegradation	4
2.1.1 Natural Attenuation	4
2.1.2 Enhanced Aerobic Biodegradation	4
2.1.3 Enhanced Anaerobic Biodegradation	5
2.1.3.1 Oxidative anaerobic biodegradation	5
2.1.3.2 Reductive anaerobic biodegradation	5
2.2 The Microbially Catalyzed Reductive Dechlorination of PCE	6
2.2.1 Electron Donors	6
2.2.2 Nutrients	7
2.2.3 Electron Acceptor Depletion	8
2.3 Hydrogeological/Geochemical Considerations	9
3.0 PRELIMINARY SITE ASSESSMENT	10
3.1 Define Project Goals	10
3.1.1 Degree of Treatment	10
3.1.2 Time Requirements/Constraints	11
3.2 Site History and Existing Data Review	13
3.3 Development of Preliminary Conceptual Model	14
3.4 Assess Site Potential	14
3.4.1 Site Rating System Instructions	15
3.4.2 Contaminant Profile	15
3.4.3 Hydrogeological Profile	17
3.4.4 Geochemical Profile	18
3.4.5 Site Rating System Results	20
4.0 TEST PREPARATIONS	23
4.1 Selection of Potential Testing Location	23
4.2 Administrative Preparations	24
4.2.1 Test Plan Preparation	24
4.2.2 Regulatory Approval of Test Plan	24
4.2.3 Application for Required Permits	24
4.2.4 Facility Clearances	25
4.3 Characterization of Potential Testing Location	25
4.3.1 Survey and Preparation of Test Area	25
4.3.2 Analytical Methods	26
4.3.3 Aquifer Core Collection Methods	27
4.3.3.1 Split-spoon sampling	27

4.3.3.2	Core-barrel sampling	28
4.3.3.3	Direct-push core sampling	28
4.3.4	Groundwater Sampling	29
4.3.5	Aquifer Testing	30
4.3.5.1	Groundwater flow direction	31
4.3.5.2	Effective porosity	31
4.3.5.3	Hydraulic conductivity	32
4.3.5.4	Groundwater velocity	34
4.4	Refinement of Conceptual Model	34
4.5	Assessment of Potential Testing Area	34
5.0	MICROCOSM STUDY	36
5.1	Importance of Site-Specific Investigations	36
5.2	Sample Collection	37
5.2.1	Soil Samples	37
5.2.2	Groundwater Samples	38
5.3	Run Studies	38
5.3.1	Microcosm Preparation	38
5.3.2	Incubation and Analyses	41
5.3.2.1	Determination of volatile organic compounds by gas chromatographic analysis of headspace samples	41
5.3.2.2	Analysis of volatile fatty acids	44
5.3.2.3	Analysis of lactate and benzoate	45
5.3.3	Additionally Recommended Microbiological Assessment (Optional)	45
5.4	Data Analysis to Determine Optimum Injection Formulation	46
6.0	FIELD TESTING	53
6.1	System Design	53
6.1.1	Distribution and Direction of the Electron Donor Feed Solution	53
6.1.2	Hydraulic Retention Time in the Testing Zone	55
6.1.3	System Alignment	56
6.1.4	Monitoring Equipment	56
6.1.5	Fouling	57
6.2	Administrative Preparations	57
6.3	Field System Installation	57
6.3.1	Drilling	58
6.3.2	Installation of Wells	58
6.3.3	Aboveground System Components	59
6.4	Field Testing Procedures	62
6.4.1	Phase I Injection Preparations	62
6.4.1.1	Determination of the initial injection flow rate	62
6.4.1.2	Estimated injectate travel time to monitoring locations	63
6.4.1.3	Select tracer and concentration	64
6.4.1.4	Determine strength of tracer stock solution and feed rate	65
6.4.1.5	Calculate the Phase I electron donor demand	66
6.4.1.6	Identify Phase I electron donor(s) and calculate desired concentration in injectate	66
6.4.1.7	Determine maximum strength of electron donor stock solution and feed rate	67
6.4.1.8	Prepare stock solutions	68

6.4.2 Phase I Injection: Tracer Testing and Electron Acceptor Depletion	68
6.4.3 Phase II Injection: Steady State System Operation	69
6.4.4 System Monitoring Protocol.....	69
6.4.5 System Maintenance	70
7.0 DATA ANALYSIS AND INTERPRETATION.....	72
7.1 Tracer Data	72
7.2 Chloroethene Data	73
7.3 Ethene and Ethane	74
7.4 Methane	74
7.5 Electron Acceptor Data	74
7.6 Final Technology Assessment.....	75
8.0 SCALE-UP CONSIDERATIONS	77
9.0 REFERENCES	79

List of Figures

Figure 1.1	RABITT Decision Flowchart	3
Figure 2.1	Reductive Dechlorination of PCE	6
Figure 2.2	Role of Hydrogen in Reductive Dechlorination	7
Figure 5.1	Results from Microcosm Studies with Subsurface Material from NAS Fallon	47
Figure 5.2	TCE Production from PCE in Two Replicate, Lactate-Fed Microcosms from NAS Fallon Study	49
Figure 6.1	Test Plot Layout	54

List of Tables

Table 2.1	Possible Reactants and Products of Specific Terminal Electron-Accepting Processes	8
Table 3.1	Contaminant Profile Scoring Table	17
Table 3.2	Hydrogeological Profile Scoring Table	17
Table 3.3	Geochemical Profile Scoring Tables	19
Table 3.4	RABITT Rating System Score Summary Table	20
Table 4.1	Analytical Methods for Examination and Testing of Aquifer Cores	26
Table 4.2	Analytical Methods for Testing of Groundwater Samples	27
Table 5.1	Conditions to be Examined in Serum Bottle Microcosm Studies	40
Table 5.2	Retention Times for Compounds from Single-Injection Gas Chromatography Analysis	43
Table 5.3	Most-Probable Number Analysis for Microbial Populations in a Groundwater Sample from NAS Fallon, Nevada	46
Table 6.1	Solubilities of Typical Electron Donor/Nutrient Formulation Components	67

List of Abbreviations

bgs	below ground surface
BTEX	benzene, toluene, ethylbenzene, and xylenes
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
cf _d	cubic foot (feet) per day
DCE	dichloroethene
DNAPL	dense, nonaqueous-phase liquid
DO	dissolved oxygen
DOC	dissolved organic carbon
DoD	U.S. Department of Defense
ECD	electron capture detector
EPA	U.S. Environmental Protection Agency
FID	flame ionization detector
fp _d	foot (feet) per day
fp _s	foot (feet) per second
GC	gas chromatograph
gpd	gallon(s) per day
gpm	gallon(s) per minute
HASP	health and safety plan
HPLC	high-performance liquid chromatography
HRT	hydraulic retention time
IC	ion chromatography
ID	inside diameter
IWTP	industrial wastewater treatment plant
K	hydraulic conductivity
LNAPL	light nonaqueous-phase liquid
MCL	maximum contaminant level
MW	molecular weight
MPN	most-probable number
NAPL	nonaqueous-phase liquid
NAS	Naval Air Station
NTU	nephelometric turbidity unit
ORP	oxidation-reduction potential
ORC	oxygen release compound
PCE	tetrachloroethene
POC	point of contact
PTFE	polytetrafluoroethene
PVC	polyvinyl chloride
QA/QC	quality assurance/quality control

RABITT	reductive anaerobic biological in situ treatment technology
RGD	reduction gas detector
RI	remedial investigation
RI/FS	remedial investigation/feasibility study
SF	safety factor
SVE	soil vapor extraction
TCE	trichloroethene
TCD	thermal conductivity detector
TEAP	terminal electron accepting process
TOC	total organic carbon
UV	ultraviolet
VC	vinyl chloride
VFA	volatile fatty acid
VOA	volatile organic analysis
VOC	volatile organic compound
YE	yeast extract

1.0 PROTOCOL OVERVIEW

1.1 Protocol Objective

This protocol provides comprehensive instructions for implementing a treatability study to determine the potential of the reductive anaerobic biological in situ treatment technology (RABITT) to enhance the reductive dechlorination of tetrachloroethene (PCE) or trichloroethene (TCE) to ethene at a specific site. It is not intended as a guide to full-scale site remediation, but rather as a tool for determining the potential to biotreat chloroethenes at a given site.

1.2 Protocol Scope

The protocol is designed to evaluate whether appropriate microbial populations and geochemical conditions exist or can be produced in situ to support biotreatment of chlorinated solvents. Successful implementation of the RABITT Test Protocol will provide qualitative, and potentially quantitative evidence to support the selection of in situ biotreatment as an appropriate remedial option, subject to limitations imposed by the site hydrogeology.

This protocol is for use at sites where the extent of contamination and the hydrogeology have been determined through a site investigation. Such information is necessary for making a preliminary assessment of the applicability of RABITT and for selecting an appropriate testing location within a delineated plume.

The protocol is not meant as a guide for designing either full or pilot-scale in situ biotreatment systems for chlorinated ethenes or any other contaminant. Appropriate selection and the successful implementation of in situ treatment systems are controlled by several site-specific parameters, including hydrology, geology, geochemistry, and microbiology. The site geology and hydrology define the boundaries for our ability to enhance or control the movement of fluids throughout a site. Numerous scenarios and approaches exist for the delivery of nutrients, and the control of mixing and transport in situ. These design decisions are best made in the context of specific site conditions and local regulatory concerns. The protocol is not meant to address these issues.

The target audiences for the protocol are base/facility environmental managers and their environmental and operations support contractors. As such, this document contains both background and

application/implementation information. The document should conceivably allow for successful implementation of the protocol by a support contractor experienced in hydrogeology and environmental engineering, as well as provide the information needed by project managers to evaluate both the test protocol (and pre-implementation work plans) and the resulting performance data.

The treatability study was developed to provide the user with an efficient way to acquire the necessary data to decide whether to exclude RABITT from further consideration or to proceed to pilot- or full-scale application. The tests contained within this protocol are designed to screen out RABITT as early in the evaluation process as possible to avoid the costs of additional characterization and pilot demonstration.

1.3 Protocol Layout

The phased approach of this protocol allows the user to screen out RABITT early in the evaluation process in order to save time and cost. The protocol guides the user through a decision process in which information is collected and evaluated to determine if the technology should be given further consideration (see Figure 1.1). RABITT will be screened out if site-specific characteristics, regulatory constraints, or other logistical problems suggest that the technology will be difficult or impossible to employ, or if a competing technology is clearly superior.

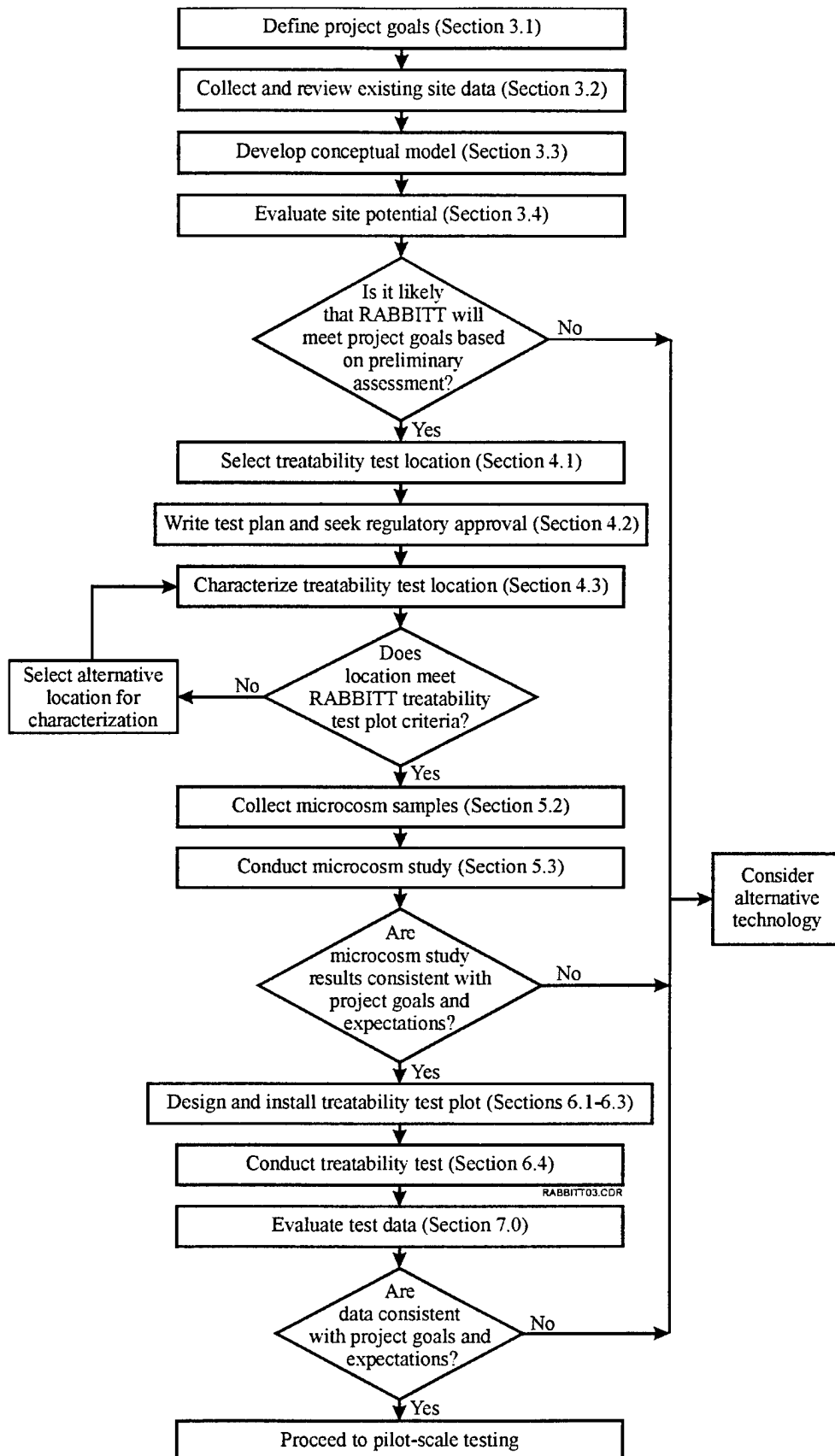


Figure 1.1 RABITT Decision Flowchart

2.0 INTRODUCTION

The widespread use of chloroethenes as solvents and in the production of chemicals and plastics has led to the inevitable release of these compounds into the environment. Accidental spills and improper storage and disposal practices have allowed these compounds to find their way into the subsurface where they have become common groundwater contaminants (Gibson et al., 1994; Sewell and Gibson, 1991). Because both PCE and TCE are stable compounds that resist aerobic degradation or require the presence of an electron-donating co-contaminant for anaerobic transformation, these compounds tend to persist in the environment. Their persistence, combined with the toxicity of all chloroethenes, frequently requires that existing contamination be actively addressed. For this reason, considerable research has been performed to develop cost-effective methods for removing these compounds from the subsurface environment.

2.1 In Situ Biodegradation.

The need for cost-effective subsurface remediation has driven the development of in situ bioremediation strategies. In situ bioremediation has gained acceptance because it can effectively treat sorbed/trapped material over large and sometimes poorly accessible areas and often in less time (Sims et al., 1992). In addition, it has the benefit of destroying organic contaminants as opposed to transferring them to another phase. Although each of these advantages makes in situ bioremediation a competitive option for subsurface treatment, several potentially process-limiting factors must be evaluated before deciding to use this technology. Sections 2.1.1 through 2.1.3 present several in situ bioremediation technologies and their specific limitations.

2.1.1 Natural Attenuation. Under favorable conditions, indigenous microorganisms may degrade contaminants in situ at acceptable rates without human intervention. This treatment option, known as natural attenuation, has recently gained regulatory acceptance for some applications. No active treatment is undertaken with the natural attenuation option, but a comprehensive monitoring system is put in place to verify contaminant destruction, examine microbial activity, and observe plume movements. Although natural attenuation may appear to be an appealing low-cost option, it is costly to monitor a slowly degrading plume of recalcitrant compounds over many decades. Therefore, full-scale natural attenuation of recalcitrant compounds may not be the most practical or cost-effective option.

2.1.2 Enhanced Aerobic Biodegradation. The most widely accepted in situ bioremediation strategies, e.g., bioventing, employ aerobic microorganisms to oxidize contaminants. The enhanced aerobic biodegradation strategies stimulate microbial activity by adding oxygen, usually through delivery of air,

into oxygen-limited subsurface environments. These technologies have proven successful for the remediation of reduced compounds such as petroleum hydrocarbons.

Aerobic cometabolism is another enhanced aerobic biodegradation strategy used for the remediation of chlorinated solvents (Hopkins et al., 1993, Roberts et al., 1990; Russell et al., 1992; Nelson et al., 1988). In addition to the delivery of oxygen, a cosubstrate is added to induce the production of oxygenase enzymes within a microbial population. Cosubstrates including methane, propane, butane, toluene, phenol, or other aromatic hydrocarbons that have shown success for supporting TCE degradation. Oxygenase enzymes are responsible for the epoxidation and subsequent destruction of contaminants such as the dichloroethenes (DCEs) and TCE.

Although sometimes effective for the treatment of TCE, enhanced aerobic strategies require large amounts of cosubstrate and are not effective against extremely oxidized compounds such as PCE. For this reason, anaerobic strategies have been developed that allow anaerobic microbial communities to capitalize on the potential energy that exists between highly oxidized contaminants such as PCE and TCE and reduced biological substrates.

2.1.3 Enhanced Anaerobic Biodegradation. Anaerobic bioremediation systems can be divided into two subsets, those that use oxidative mechanisms to destroy contaminants, and those that use reductive mechanisms.

2.1.3.1 Oxidative anaerobic biodegradation. Just as aerobic biodegradation systems utilize oxygen as a terminal electron acceptor to stimulate microbial activity, oxidative anaerobic systems require other terminal electron acceptors, such as nitrate, to stimulate biodegradation. The contaminant serves as the electron donor, and in most instances allows the microorganism to derive useful amounts of energy from the reaction. These systems work most efficiently with reduced contaminants, such as hydrocarbons, but biodegradation rates are typically slower than their aerobic counterparts.

2.1.3.2 Reductive anaerobic biodegradation. While in oxidative anaerobic systems the contaminant is used as an electron donor, in reductive systems highly oxidized contaminants (e.g., PCE) are used as electron acceptors. RABITT is a process that attempts to stimulate this reductive pathway. The process begins by supplying excess reduced substrate (electron donor) to a microbial consortium, i.e., a cooperative community of microbial species. The presence of the substrate expedites the exhaustion of any naturally occurring electron acceptors. As the natural electron acceptors are depleted,

microorganisms capable of discharging electrons to other available electron acceptors, such as oxidized contaminants, gain a selective advantage. The intricacies of these microbial communities are complex, but recent research has provided some insight into methods for enhancing populations of contaminant-degrading microorganisms.

2.2 The Microbially Catalyzed Reductive Dechlorination of PCE.

The reductive dechlorination of PCE to ethene proceeds through a series of hydrogenolysis reactions (see Figure 2.1). Each reaction becomes progressively more difficult to carry out; subsequently, the DCEs, particularly *cis*-DCE, and vinyl chloride (VC) tend to accumulate in anaerobic environments.

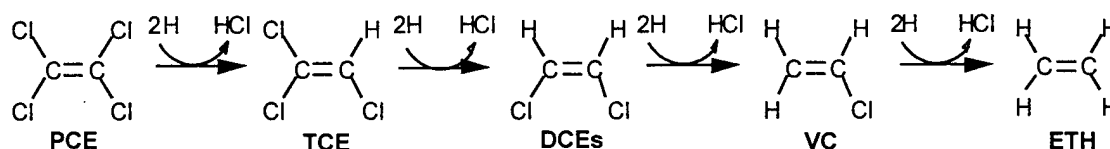


Figure 2.1. Reductive Dechlorination of PCE

2.2.1 Electron Donors. The selection of an appropriate electron donor may be the most important design parameter for developing a healthy population of dechlorinating microorganisms. Recent studies have indicated a prominent role for molecular hydrogen (H_2) in the reductive dechlorination of chloroethenes (Holliger et al., 1993; DiStefano et al., 1992; Maymó-Gatell et al., 1995; Gossett et al., 1994; Zinder and Gossett, 1995). Most known dechlorinators can use H_2 as an electron donor, and some can use only H_2 . Because more complex electron donors are broken down into metabolites and residual pools of H_2 by other members of the microbial community, they may also be used to support dechlorination (see Figure 2.2) (Fennell et al., 1997; Smatlak et al., 1996; DiStefano et al., 1992).

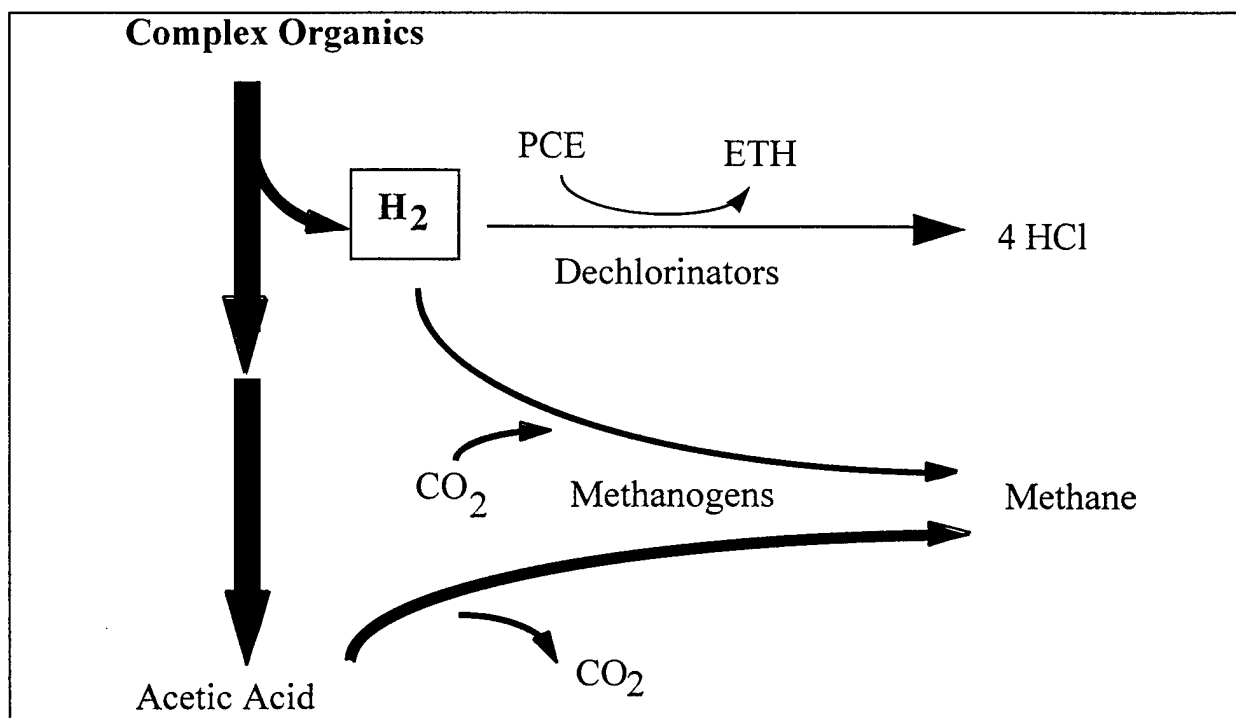


Figure 2.2 Role of Hydrogen in Reductive Dechlorination

The rate and quantity of H_2 made available to a degrading consortium must be carefully engineered to limit competition for hydrogen from other microbial groups, such as methanogens and sulfate-reducers. Competition for H_2 by methanogens is a common cause of dechlorination failure in laboratory studies. As the methanogen population increases, the portion of reducing equivalents used for dechlorination quickly drops and methane production increases (Gossett et al., 1994; Fennell et al., 1997). The use of slowly degrading nonmethanogenic substrates will help prevent this type of system shutdown and allow a larger zone of treatment in the subsurface.

2.2.2 Nutrients. In addition to proper electron donor selection, nutrient availability may be a critical factor in maintaining a healthy dechlorinating consortium. In one instance, attempts to isolate a microbial species responsible for dechlorination led to the discovery that nutritional factors probably had been supplied by other consortium members. Highly enriched dechlorinating cultures required the addition of vitamin B_{12} and sludge supernatant to sustain dechlorination (Maymó-Gatell et al., 1995). Speculation exists that acetogens may supply the unknown nutritional factors required by the dechlorinating organism(s) (DiStefano et al., 1992). Fortunately, in situ applications support a variety of microbial species. This microbial diversity, combined with the addition of nutritional (vitamin) supplements, should support a dechlorinating microbial community.

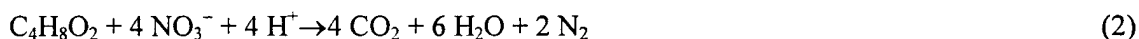
2.2.3 Electron Acceptor Depletion. The successful application of RABITT depends upon the depletion of electron-accepting chemical species. The most environmentally relevant species include O_2 , NO_3^- , $Mn(IV)$, $Fe(III)$, and SO_4^{2-} . When evaluating a site for RABITT applicability, one must investigate the relative abundance of these compounds in both the groundwater and the aquifer solids. Although aqueous-phase acceptors such as O_2 and NO_3^- take primary consideration, it is imperative that the aquifer solids be characterized because they can serve as a reservoir of relatively insoluble electron-accepting species such as $Fe(OH)_3$ or $CaSO_4$. Once the electron-accepting species have been quantified, the amount of electron donor required to deplete them can be estimated by evaluating the stoichiometric relationship between the selected electron donor and each electron acceptor present on site. Higher levels of electron acceptor require more electron donor and therefore raise treatment costs. A series of generic reactions are given in Table 2.1 to illustrate some of the possible reactants and products.

Table 2.1 Possible Reactants and Products of Specific Terminal Electron-Accepting Processes

Predicted Reaction	Process
Electron donor + $O_2 \rightarrow CO_2 + H_2O$	Aerobic respiration
Electron donor + $NO_3^- \rightarrow CO_2 + H_2O + N_2$	Denitrification
Electron donor + $MnO_2 \rightarrow MnCO_3 + Mn(OH)_2$	Manganese reduction
Electron donor + $FeOOH \rightarrow FeCO_3 + Fe(OH)_2$	Iron reduction
Electron donor + $SO_4^{2-} \rightarrow H_2S + CO_2 + H_2O$	Sulfate reduction

Depletion of subsurface electron acceptors should effectively eliminate the competition for reducing equivalents between dechlorinators and such groups as nitrate reducers, iron reducers, and sulfate reducers. Competition from methanogens, on the other hand, may never be eliminated, so it must be managed by the choice and delivery of electron donor.

Once an electron donor has been selected and the electron acceptors have been characterized, the stoichiometric relationship between them can be determined. An equation for each electron acceptor present at the site must be balanced using the selected electron donor. Once balanced, the molar ratio of donor to acceptor can be determined from the equation. For example, a site containing oxygen, nitrate, and manganese oxides would yield the following equations if butyric acid ($C_4H_8O_2$) were being used as an electron donor:



Equation 1 stipulates that 1 mole of butyric acid can reduce 5 moles of oxygen to carbon dioxide and water. Similarly, Equation 2 demonstrates that 1 mole of butyric acid can reduce 4 moles of nitrate to nitrogen gas. These molar ratios represent an ideal case where the entire electron donor dosage is used to reduce the electron acceptor present in the treatment zone. When calculating the actual electron donor dosage, a safety factor must be incorporated to account for uncharacterized electron sinks and the advective transport of electron acceptors into the treatment zone. Site-specific conditions such as groundwater flow rate, surrounding electron acceptor concentrations, depth to the water table, rainfall frequency, and level of site characterization will influence the selection of the safety factor.

Because treatment alternatives and budgetary constraints are different for each site, no rule of thumb exists for screening sites based on electron acceptor concentrations. The required mass of electron donor should be estimated so its cost can be calculated. Afterwards, a site-specific cost benefit analysis must be undertaken to determine if the site is a good candidate for RABITT application.

2.3 Hydrogeologic/Geochemical Considerations

The geologic setting in which a RABITT system is installed governs its successful operation. RABITT systems rely on the delivery of dissolved amendments throughout a contaminant plume; administering these amendments through both the vertical and horizontal extent of contaminant plumes sounds deceptively easy, but requires careful engineering and a knowledge of the geologic parameters affecting groundwater flow and transport. The subsurface composition, aquifer properties, and groundwater characteristics each exert a direct influence over RABITT feasibility. The subsurface composition and resulting aquifer properties dictate groundwater movement and consequently affect the transport of contaminants and RABITT groundwater amendments. Similarly, groundwater characterization reveals information about dynamic subsurface chemical interactions. Each of these factors should be investigated so the performance potential of RABITT can be evaluated.

3.0 PRELIMINARY SITE ASSESSMENT

This section is designed to assess the applicability of RABITT to a specific site. The following subsections discuss what project-specific questions to consider, what information to look for, and how that information can be used to assess RABITT feasibility. The first phase of the assessment process is defining project goals and reviewing pertinent literature to evaluate RABITT's potential for achieving those goals. The second phase requires the compilation and evaluation of relevant site history and existing data so a site's overall potential may be assessed and a conceptual model developed. Finally, a numerical site rating system is provided to help the protocol user assess the applicability of RABITT under prevailing site conditions. By the end of this section the reader should feel comfortable deciding whether to proceed with RABITT treatability testing at a specific site.

3.1 Define Project Goals

Defining project goals, including the target cleanup level, time constraints and cost is the first step in assessing RABITT's suitability for implementation at a particular site. RABITT will be eliminated from further consideration at this stage of the assessment process if its ability to achieve prescribed project goals is doubtful, thereby saving the expense of conducting a treatability study.

Because RABITT is an emerging technology, quantitative analyses of its abilities and limitations have yet to be firmly established. The following subsections provide a cursory discussion of potentially achievable treatment levels and factors affecting treatment times by briefly reviewing published laboratory and field results and their implications. This type of information will be used to make a preliminary estimate of RABITT's potential treatment performance; treatability testing will provide site-specific treatment performance data and allow refined cost estimations based on electron donor demand and treatment rate.

3.1.1 Degree of Treatment. Laboratory results suggest that the use of RABITT to clean up chloroethenes to below detection levels is possible and can be achieved at a cost that is at least competitive with conventional technologies, if not lower. Demonstrations of PCE and TCE removal in anaerobic microcosms are common, but often the process ends with the accumulation of DCEs and VC, a known human carcinogen (Bradley and Chapelle, 1996; Holliger et al., 1993; Parsons et al., 1984; Gibson et al., 1994; Gibson and Sewell, 1992). Although VC is more easily degraded by aerobic microorganisms, its destruction by anaerobic microcosms has been observed (Tandoi et al., 1994; Major

et al., 1995; de Bruin et al., 1992; DiStefano et al., 1991; Freedman and Gossett, 1989). Although lab results have been promising, in situ application can yield vastly different outcomes. The treatability test described in this protocol is designed to elucidate these differences. Nonetheless, the potential for complete in situ dechlorination of PCE, TCE, DCEs, and VC to ethene does exist. The regulatory and administrative criteria defining success should be outlined before proceeding with the site screening so borderline sites may be excluded from consideration when stringent objectives are required.

3.1.2 Time Requirements/Constraints. The total treatment time for an in situ effort will encompass the time it takes to deplete available electron acceptors, acclimatize a healthy population of dechlorinating microorganisms, and allow the dechlorination reaction to proceed to its conclusion. Site-specific conditions will influence the total time required for treatment; for instance, methanogenic sites exhibiting some level of dechlorination should require considerably less time to demonstrate enhanced dechlorination than sites with aerobic groundwater and no evidence of dechlorination. Other factors influencing the time required to treat a site include aquifers with low hydraulic conductivities, which will require more time for delivery of substrate throughout the subsurface, and the presence of dense, nonaqueous-phase liquid (DNAPL), which would require considerably longer treatment times due to the rate limitation imposed by dissolution.

The time required for electron acceptor depletion depends on the electron donor supply and utilization rate, and on initial electron acceptor concentrations and the rate they are replenished by groundwater flow and recharge events. The large number of variables affecting electron acceptor depletion makes it difficult to predict, but a recent study of a petroleum hydrocarbon-contaminated aquifer (up to 4.5 mg/L benzene, toluene, ethylbenzene, and xylenes [BTEX]) may provide insight into terminal electron accepting process (TEAP) shifts. Researchers have observed time lags from less than 10 days to about 3.5 months to shift from one TEAP to another, i.e., from sulfate reduction to methanogenesis (Vroblesky and Chapelle, 1994). No amendments were added to the subsurface, so all shifts were naturally occurring. A well-designed RABITT system should efficiently reduce the time required to shift the TEAP by providing a steady supply of an ideal substrate.

The onset of dechlorinating activity may not begin immediately after depletion of natural electron acceptors. Delays may result from the development and acclimation of a healthy population of dechlorinating microorganisms. Unfortunately, data describing this process and its time requirements are scarce. In one study, a first-generation microcosm inoculated from a laboratory anaerobic digester began dechlorinating PCE in about 2 weeks (Freedman and Gossett, 1989). The microbial community

found in the subsurface at each site will be significantly different from the culture in this study, even if highly reducing conditions have been established. In a separate study using chlorinated ethene-contaminated aquifer material, the reductive dechlorination of PCE became evident after 51 days of incubation (Gibson et al., 1994). Realistically, lag periods exceeding 2 months from electron acceptor depletion should be expected.

Once dechlorination has begun, the time required to completely dechlorinate chloroethenes in situ will depend on site-specific conditions. In one study, under ideal laboratory conditions, the production of ethene from PCE began in a matter of hours, with ethene accounting for 99% of dechlorination products after 4 days (Tandoi et al., 1994). Ideal conditions include an enriched dechlorinating culture in well-mixed fluid with controlled PCE and electron donor concentrations at 35°C. Obviously, these conditions will never be met in situ, and a laboratory study conducted under less ideal conditions, i.e., nonenriched culture at 10°C, has shown a substantially slower dechlorination rate. This study used aquifer microcosms amended with 1.6 mg/L of PCE, and did not begin to demonstrate ethene production until after day 46 (Major et al., 1995). In the same investigation, depletion of VC was observed after 145 days. Reviewing the literature for in situ studies at sites with hydrogeological and geochemical conditions similar to those at a given site may provide some insight into the overall dechlorination rate one might expect, but cannot supply the invaluable information gleaned from site-specific treatability testing.

When considering the time required to implement a RABITT system, one should include a minimum of 6 months for RABITT treatability testing. This 6-month testing time frame assumes 1 month for electron acceptor depletion, 2 months for acclimation, and 3 months for evaluating treatment data. A minimum of 3 months should be allotted for the treatment phase because shorter periods may not produce the data required to thoroughly evaluate RABITT potential at a specific site.

Based on published information and a careful stepwise approach, full-scale implementation of RABITT will require several years to complete. Sites with existing groundwater manipulation systems (e.g., pump and treat) that may be used to distribute the electron donor may require less time, while sites with stringent treatment goals may require more. If a project's goals include time constraints that require very rapid and easily predictable cleanup benchmarks, then RABITT may not be a suitable technology. Unfortunately, few quick remediation alternatives exist, and those that do act quickly are apt to be very expensive.

3.2 Site History and Existing Data Review

If RABITT meets the constraints set forth by project goals, screening based on chemical, biological, and hydrogeological characteristics can begin in earnest. The site history and data review will be initiated once the project manager identifies the potential facilities and specific sites where the technology may be applicable. The project manager will also provide a contact person at each facility (hereafter called the facility point of contact (POC)). The project manager and/or the facility POC will supply any relevant documents (site characterization reports, underground utility drawings, remedial investigation/feasibility studies [RI/FS], etc.) pertaining to the contaminated area.

The contractor should request documents containing information about chemicals stored, used, or transferred on site, as well as any previous land uses. In addition, potentially relevant climatic parameters, such as historical rainfall amounts and temperature ranges, should be researched, as should any previous environmental characterization and/or remediation work performed on site. The amount of existing data available will vary between sites; data describing site contamination and hydrogeology will prove particularly valuable, if available. Finally, nearby groundwater wells and discharge points should be identified to determine possible sampling locations and/or exposure pathways. The list of review topics given below outlines the information crucial to the planning of a treatability test and the development of a preliminary conceptual model.

- Site History
 - Chemicals stored, used, or transferred on site
 - Previous land uses
 - Climatological history
 - Previous remedial activity (i.e., RI/FS)
 - Topographic maps
 - Underground utilities
- Contaminant Data
 - Inventory (which contaminants are present?)
 - Identification of source
 - Three-dimensional spatial distribution
 - Phase distribution (i.e., dissolved, sorbed, vapor, or nonaqueous-phase liquid [NAPL])
 - Temporal distribution (historical water quality data showing contaminant concentration through time)
- Hydrogeology
 - Aquifer composition, stratigraphy, depth, heterogeneity
 - Hydraulic conductivity
 - Hydraulic gradient
 - Groundwater velocity

- Groundwater wells and discharge points
- Geochemistry
 - Dissolved gases (oxygen, methane, hydrogen sulfide)
 - Dissolved and solid-phase electron-accepting species (NO_3^- , Fe(III), Mn(IV), SO_4^{2-})
 - Dissolved organic carbon
 - Groundwater pH, alkalinity, redox potential and temperature

In instances where few data are available, additional site characterization activities will be required before the development of a conceptual model or the effective screening of remedial options may begin. In this case, as much information as possible should be collected to determine where data deficiencies exist, and a sampling plan should be devised to address them.

3.3 Development of Preliminary Conceptual Model

After gathering the site history and existing data, a preliminary conceptual model should be developed. Existing data should be compiled into a detailed three-dimensional representation of the site contamination and hydrogeology. Model construction involves superimposing data contours on topographic site maps and creating cross-sectional representations of the contaminated subsurface. This process will elucidate data deficiencies and prompt the planning of future site-characterization activities, including the spacing of monitoring wells and the development of a sampling and analysis plan. Often this information has been compiled in the form of a remedial investigation (RI) report. In such cases, the existing model should be thoroughly examined to gain an understanding of site contamination, geochemical, and hydrogeological conditions.

3.4 Assess Site Potential

This section provides background information on the implications of site data. This information should allow the user to evaluate a site in the context of specific project goals and regulatory concerns. In conjunction with this information, a generic site rating system is provided. This system numerically rates a site's potential for success using available data. Site contamination, hydrogeology, and geochemistry are discussed and evaluated. Whenever possible, subjective and qualitative assessments of site characteristics have been intentionally excluded. Although sites scoring well should, in general, be less expensive to implement, explicit cost data has also been excluded from the rating system to accommodate a wide variety of site conditions and the potentially vast differences in project goals and budgets. Results generated from the rating system serve only as an indication of potential for

stimulating microbially catalyzed reductive dechlorination at a specific site. They are not intended nor should they be used as a substitute for a thorough site-specific evaluation or treatability testing.

This rating system assumes that the parent compound is either PCE or TCE and that only the most recent data are being used. In addition, different areas within the plume will have different contaminant, geochemical, and hydrogeological profiles, and therefore the potential for stimulating or accelerating dechlorination will be variable. For this reason it is recommended that the average or prevailing conditions within a plume be used for the purposes of this screening.

3.4.1 Site Rating System Instructions. The rating system is broken into three independent categories, the contaminant profile (Table 3.1), the hydrogeological profile (Table 3.2) and the geochemical profile (Table 3.3). Each begins with a brief discussion of the category followed by a category-scoring table. Category-scoring tables list a set of possible site conditions followed by an assigned score. Select the description that best characterizes the site of interest and record the listed score in Table 3.4. After selecting one description from each scoring table, sum the list of scores in Table 3.4 to come up with a total or overall score. Total scores may then be compared with the site potential analysis given in Section 3.4.5. This section provides an assessment of site potential based on total point value ranges.

3.4.2 Contaminant Profile. The primary consideration when selecting an appropriate remediation strategy is the type and extent of site contamination. An inventory of contaminants, their concentrations, and their distribution throughout the site will be important for determining the feasibility of implementing RABITT. The presence, relative concentration, and distribution of chloroethene daughter products will be particularly important when assessing sites for dechlorination potential. For most sites, the magnitude and extent of contamination has already been assessed during the RI; sites with existing data deficiencies should be characterized before proceeding with RABITT or any other remedial treatment option.

Because the presence of co-contaminants may significantly affect RABITT treatment effectiveness, the site contamination assessment should begin with an inventory of site contaminants. Co-contaminant impacts may be either beneficial or detrimental. For instance, high concentrations of heavy metals or other highly toxic organic compounds may impede microbial activity and decrease RABITT treatment efficiency. Conversely, some organic co-contaminants (e.g., petroleum hydrocarbons) can serve as electron donors and help drive the depletion of in situ electron acceptors and promote dechlorination. In one such case, a chemical transfer facility in North Toronto contaminated with PCE, methanol, and

acetate demonstrated in situ reduction of PCE to ethene without the addition of a supplemental electron donor (Major et al., 1995). Recognizing co-contaminants and their influence on microbial activity will help prevent the application of RABITT at unfavorable sites and will permit designers to take advantage of alternative electron donors already in the groundwater.

In addition to the effects of unrelated (nonhomologous) co-contaminants, the presence or absence of dechlorination daughter products provides insight into a site's dechlorination potential. For instance, if the site history review reveals that only PCE was used on site, the presence of its daughter products would strongly suggest that reductive dechlorination is already taking place in situ. This activity demonstrates the site's dechlorination potential and makes it a good candidate for RABITT. On the other hand, the absence of these daughter products does not necessarily preclude RABITT application, but provides a good indication that aerobic or other adverse conditions may predominate at the site. If it is determined that groundwater conditions are indeed anaerobic and dechlorination daughter products are absent, other possible rate-limiting factors (e.g., high nitrate concentration or extreme pH value) must be examined before proceeding with RABITT.

A complete contaminant profile includes a description and evaluation of contaminant distribution. The contaminant distribution analysis should include a depiction of plume dimensions, concentration contours, and phase partitioning. A spatial description of the plume and its concentration contours can help delineate the contaminant source, groundwater flow direction, and plume movement. Concentration contours may be used to estimate the total quantity of contaminant, predict microbial toxicity, and aid in the placement of monitoring equipment and system components. In addition, persistent concentrations at or above one percent of a contaminant's solubility suggest the potential for NAPL pockets that may limit bioavailability. If DNAPL is known or suspected to exist, RABITT could still be a low-cost substitute for long-term containment pumping. Plume concentration and dimension data help customize treatment and sampling components to a site, and knowledge of contaminant phase distribution helps screen the potential treatment alternatives.

Determining which phases and in what proportions the bulk of contamination has partitioned will allow an evaluation of RABITT applicability. Because RABITT delivers electron donors and nutrients to the groundwater, it offers little or no treatment of vadose zone contamination. Consequently, RABITT would be an imprudent remedial option at a site with high levels of vadose zone contamination and little or no aqueous-phase contamination. Similarly, highly localized releases with high contaminant

concentrations may be more effectively cleaned up with a physical/chemical technology than by RABITT.

Table 3.1 Contaminant Profile Scoring Table

Evidence of Daughter Product Formation	Score
Ethene detected above background levels in or immediately downgradient of a VC daughter plume	25
VC daughter plume associated with or immediately downgradient from a DCE daughter plume	15
DCE daughter plume associated with or immediately downgradient from the parent plume (PCE or TCE)	5
No daughter products present and greater than 1 mg/L O ₂	0
No daughter products, dissolved oxygen less than 0.5 mg/L, and nitrate less than 1 mg/L	-6

3.4.3 Hydrogeological Profile. The success of RABITT depends upon the effective distribution of electron donor and nutrients throughout the subsurface, consequently the ability to control the movement of groundwater is imperative. The large point value assigned to hydraulically conductive aquifers in Table 3.2 reflects this necessity. Sites with a hydraulic conductivity less than or equal to 10^{-5} cm/sec have been assigned an exceptionally low score to supersede any other positive site conditions. At such sites, the difficulties in distributing electron donor through the subsurface would make implementation of RABITT infeasible even under the most promising microbiological and geochemical conditions.

Because many hydrogeological parameters are subjective (e.g., stratification), and because an averaged hydraulic conductivity accounts for several other hydrogeological variables, hydraulic conductivity is the only criterion used for the purposes of numerically rating a site's hydrogeologic potential. Other less quantifiable factors (e.g., stratigraphy) must be defined and accommodated before RABITT implementation begins.

Table 3.2 Hydrogeological Profile Scoring Table

Hydraulic Conductivity, K	Score
$K \geq 10^{-3}$ cm/sec	25
$10^{-4} < K < 10^{-3}$ cm/sec	0
$K \leq 10^{-5}$ cm/sec	-50

The careful evaluation of hydrogeological data requires consideration of the data source, commonly monitoring wells. Monitoring wells typically are spaced sparsely throughout a site to allow coverage and usually are constructed with long well-screen intervals. The resulting data gathered from core logs, pumping or slug tests, or laboratory tests on undisturbed core samples taken from these wells provide information about the macroscopic hydrogeologic conditions at a site. Consequently, values obtained during a full-scale site investigation for hydrogeologic parameters often are averaged over relatively large areas or depths. Although these data give an overview of the entire site, they cannot provide the level of specificity required for the relatively small-scale RABITT treatability test; nonetheless, these data can be used to assess a site's potential for RABITT application. At promising sites, these data can also be used to select potential test plot locations.

3.4.4 Geochemical Profile. Geochemistry influences the potential for stimulating and maintaining microbially catalyzed reductive dechlorination by affecting the microorganisms responsible for catalyzing the reaction. These microorganisms require highly reducing conditions, which are manifested by depleted electron acceptor concentrations, low redox potential measurements, and the production of hydrogen sulfide or methane gas. In addition, geochemical parameters like groundwater pH, alkalinity, temperature, and dissolved organic carbon can affect the health and stability of dechlorinating microorganisms.

The geochemical profile used in this rating system applies the most recent existing data to provide a snapshot of geochemical conditions and indicate the chances of successfully promoting microbially catalyzed reductive dechlorination within a plume. Although data from outside the projected treatment plume are not intended to be used with this rating system, they can suggest what geochemical shifts, if any, have resulted from the introduction of contaminant into the groundwater. For instance, the contaminant plume may be anaerobic while the surrounding groundwater contains 2.5 mg/L of oxygen. This implies that one of the contaminants in the plume is serving as an electron donor and may help drive the depletion of electron acceptors and subsequently dechlorination.

Although potentially important sinks for reducing equivalents, manganese (IV) and iron (III) oxides and hydroxides (e.g., MnO_2) have been excluded from the rating system because of the difficulties in determining their bioavailability. Investigating the total iron and manganese concentrations in an aquifer (both solid and dissolved phase) and the distribution between Fe(II) and Fe(III) and Mn(II) and Mn(IV) is encouraged, but the results are not easily applied to a generalized site rating system. One

purpose of the RABITT treatability test is to provide information on the electron donor demand resulting from bioavailable manganese (IV) and iron (III) oxides and hydroxides.

The maximum total point value in this category is 25 points; the minimum is -16. In general, geochemical scores greater than 9 are considered favorable and scores less than -9 are considered unfavorable. Values in between, considered questionable, may permit the stimulation of biologically catalyzed reductive dechlorination, but would require geochemical manipulation (e.g., electron acceptor depletion). Low values tend to indicate higher electron acceptor concentrations, while larger values demonstrate electron acceptor deficiencies but otherwise hospitable geochemical conditions.

Table 3.3 Geochemical Profile Scoring Tables

(Use average or prevailing conditions within the selected location)

Dissolved Oxygen	Score
< 0.5 mg/L	3
0.5-1.0 mg/L	1
1.0-3.0 mg/L	0
>3.0 mg/L	-3

Nitrate	Score
< 1 mg/L	3
1-2 mg/L	1
2-5 mg/L	0
> 5 mg/L	-3

Hydrogen Sulfide	Score
> 0.1 mg/L	3
≤ 0.1 mg/L	0

Sulfate	Score
< 20 mg/L	2
> 20 mg/L	0

Redox Potential	Score
< -200 mV	1
-200 mV- 200 mV	0
> 200 mV	-1

Temperature	Score
> 15°C	3
10°C – 15°C	0
< 10°C	-3

Dissolved Organic Carbon	Score
> 20 mg/L	3
10-20 mg/L	1
< 10 mg/L	0

Bicarbonate Alkalinity	Score
> 5 g/L	1
1-5 g/L	0
< 1 g/L	-1

pH	Score
6.5-7.5	3
6.0-6.5 or 7.5-8.0	0
5.0-6.0 or 8.0-9.0	-1
< 5.0 or > 9.0	-5

Methane	Score
> 0.1 mg/L	3
≤ 0.1 mg/L	0

3.4.5 Site Rating System Results. Complete Table 3.4 with the values selected from Tables 3.1 through 3.3 and calculate a total point value. Seven site potential analyses are given below; use the total point value calculated in Table 3.4 to find the analysis with the appropriate score range. These analyses are intended to help guide protocol users in a preliminary decision making process, but they should be used in the context of all available site information. The protocol user must use rating system results in light of all available site information because the rating system is very general in nature and not designed to account for unusual or erratic site conditions.

Table 3.4 RABITT Rating System Score Summary Table

Rating Parameter	Score
Contaminant Profile	
Hydraulic Profile	
Geochemical Profile	
Dissolved Oxygen	
Nitrate	
Hydrogen Sulfide	
Sulfate	
Redox potential	
Temperature	
Dissolved Organic Carbon	
Bicarbonate Alkalinity	
PH	
Methane	
Total Point Value	

Score from 66-75: Highest Potential for Success.

Dechlorination is proceeding to ethene under favorable hydrogeological and geochemical conditions. This site is probably a good candidate for natural attenuation, but if site specific conditions warrant an accelerated rate of dechlorination because the plume poses a risk to a potential receptor, RABITT will likely be successful in achieving that goal. If RABITT will be used for this purpose, proceed to microcosm and treatability testing to determine electron donor demand and the optimum electron donor/nutrient formulation.

Score from 56-65: Promising

Dechlorination is proceeding to VC under favorable hydrogeological and geochemical conditions. The application of RABITT at such sites will likely accelerate the conversion of PCE, TCE and DCE to VC. The conversion of VC to ethene may be possible if electron donor or nutrient limitations are responsible

for the lack of degradation. If the VC daughter plume borders or enters an aerobic groundwater zone, the aerobic degradation of VC to CO₂ should be investigated. Microcosm and treatability testing should be used to assess electron donor demand, determine the optimum electron donor/nutrient formulation, and evaluate the possible conversion of VC to ethene.

Score from 36-55: Satisfactory

Some level of dechlorination is probably occurring and hydrogeological and geochemical conditions are favorable. Under these conditions, accelerating dechlorination with RABITT is likely, but care needs to be taken to ensure that DCE does not accumulate. Proceed to microcosm testing and evaluate the microbiological potential for complete dechlorination to ethene. If results are congruent with project goals, proceed with in situ treatability testing to determine the extent of chloroethene degradation and to assess electron donor demand.

Score from 16 to 35: Marginal

Although scores in this range are not strong indicators of success, they do indicate that it is worthwhile to conduct the RABITT treatability test. Sites in this range exhibiting dechlorination and favorable geochemistry often have a hydraulic conductivity in the 10⁻⁴ cm/sec range. If groundwater manipulation is plausible (i.e., $K \geq 5 \times 10^{-4}$ cm/sec), such a site should be considered favorably. Microcosm and treatability testing will provide insight into the extent of dechlorination and electron donor demand that may be expected in situ. Based on results from these studies, a cost benefit analysis should be used to see if full-scale RABITT implementation is likely to meet project goals at acceptable costs.

Score from -5 to 15: Questionable

Scores falling in this range require a closer look. Although stimulating dechlorination at this site may be possible, it will probably be more expensive and time consuming than sites with higher rankings. To be worthy of further consideration, this site must allow the effective distribution of electron donor and nutrients. Therefore, the site's hydraulic conductivity is the key. If substantial groundwater manipulation is plausible (i.e., $K \geq 5 \times 10^{-4}$ cm/sec), microcosm testing to evaluate in situ electron donor demand and the microbiological potential for dechlorination should be undertaken. If difficulties are anticipated with groundwater manipulation, the site should be excluded from further consideration.

Score from -6 to -15: Unfavorable

The combination of unfavorable geochemical conditions and questionably low hydraulic conductivity make the implementation of RABITT at this site extremely risky. Stimulating dechlorination will

probably require considerable effort to modify existing geochemical conditions, including the exhaustion of several electron -accepting species. Questionable hydraulic conductivities in the range of 10^{-4} cm/sec may significantly increase the difficulty and expense of distributing electron donor and nutrients throughout the site.

Score from - 16 to - 66: Prohibitive

Scores in this range have a hydraulic conductivity less than or equal to 10^{-5} cm/sec and as such should be excluded from further consideration. Difficulties distributing electron donor through the subsurface would make implementation of RABITT infeasible even under the most promising microbiological and geochemical conditions.

4.0 TEST PREPARATIONS

The decision to proceed with treatability testing must be followed by appropriate preparations. First, a potential testing location will be identified based on existing data and the site conceptual model. This location will undergo small-scale but detailed characterization, so a site-specific test plan will be written and submitted for regulatory approval. Once approval has been granted, applications for required permits and facility clearances will be submitted. After all approvals, permits and clearances are in place, characterization activities will begin on the potential test location. Characterization results will be used to select the final testing location.

4.1 Selection of Potential Testing Location

Typically, site characterization data found in documents like RI reports provide a macroscopic look at site conditions. It would be highly unusual for this information to be detailed enough to permit the proper design and installation of a treatability test system. Consequently, existing data will be used to select a potential testing location and to direct more detailed characterization activities at that location. These characterization activities will be discussed in Section 4.3. The following technical criteria will be used for selecting potential testing locations:

- Contaminant concentrations will be at least two orders of magnitude greater than the contaminant's detection limit, but below levels indicative of DNAPL contamination (approximately 1% of the contaminant's solubility limit). The presence of DNAPL pockets would likely affect observable reductions in parent compound and produce misleading data.
- The hydraulic conductivity in the proposed treatment zone will be $> 10^{-4}$ cm/sec.
- Groundwater velocities between 0.2 ft/day and 1.0 ft/day will be preferred, as will areas with relatively constant and predictable groundwater flow.
- Relatively homogeneous areas or zones with well-defined stratigraphy will be preferred.

Other considerations that play a pivotal role in the selection of a potential testing location will include the availability of electrical power, conflicts with infrastructure (e.g., buried utilities), the site's accessibility or remoteness, or other factors that influence a location's desirability. Because the system may require the disposal or discharge of significant quantities of extracted groundwater, proximity to a sanitary sewer line or industrial wastewater treatment plant (IWTP) should be considered.

4.2 Administrative Preparations

4.2.1 Test Plan Preparation. Before fieldwork can begin, a detailed site investigation test plan must be prepared. The project manager will provide the appropriate format for the required test plan to the contractor. If necessary, the plan will be prepared following the guidelines contained in the EPA *Guide for Conducting Treatability Studies Under CERCLA* (EPA/540/2-89-058) (1989). Following these guidelines can help to expedite the review and approval process. The plan must describe the sample collection methods and handling procedures, and list the sample analytical methods. A map showing the locations of the test areas and the sampling locations should be included. A quality assurance/quality control (QA/QC) plan, defining the data quality objectives, and a health and safety plan (HASP) should be appended to the test plan. After completion, a copy of the test plan will be distributed to all parties involved with a given site.

4.2.2 Regulatory Approval of Test Plan. Once the test plan is completed, it is necessary to obtain facility approval before submitting the plan to state and federal regulatory agencies. Typically, military bases require a 30-day review period; however, the contractor should negotiate the length of the review period with the facility POC. Final approval will be obtained from the facility after the review comments have been satisfactorily addressed. After securing facility approval, the work plan is submitted to the appropriate state and federal regulatory agencies for their review. The time for regulatory agency review can vary between 30 and 120 days. Final approval will be granted once regulatory concerns and comments are addressed. Once final regulatory approval has been obtained, preparations for field mobilization can begin.

4.2.3 Application for Required Permits. As soon as the project manager identifies a candidate site, applications must be submitted for the required permits. Obtaining permits frequently is the greatest source of delay in this type of fieldwork. Delegating the responsibility for obtaining permits early, clearly and firmly will help avoid delays caused by misunderstandings. Types of permits that may be required include the following:

- Dig/drill permit
- Groundwater extraction and injection permit
- Water discharge permit
- Vapor discharge permit.

The contractor should work with the facility POC when applying for the required permits. The contractor should not contact regulatory agencies without project manager and facility POC approval. In many cases, the project manager or facility POC will handle regulatory contacts, if they are necessary.

4.2.4 Facility Clearances. The contractor will coordinate with the facility POC to obtain access and necessary clearance to conduct the tests at the candidate test area. The contractor will arrange with the facility for the utilities (e.g., electricity and water) needed to execute the tests.

The contractor will coordinate with the facility POC to arrange for any necessary security clearances or badges. As early as possible, the contractor will supply the facility POC with a list of all personnel who will be on facility, including name, social security number, place and date of birth, and expected arrival and departure dates. The contractor staff will be responsible for securing facility passes from the pass and identification office upon arrival at the facility.

4.3 Characterization of Potential Testing Location

Preparing for the characterization of a potential testing location involves a cursory survey of the selected location, and the selection of appropriate drilling, aquifer sampling, and groundwater sampling methods. Although surveying for potential obstacles and hazards is straightforward, the variety of drilling and sampling techniques available requires thorough planning. Because site conditions will dictate the most appropriate drilling and sampling methodologies, no one method can be specified that would work at every site. For comprehensive information on site characterization and sampling techniques see the American Society for Testing and Materials (ASTM) Method D 5730, *Standard Guide for Site Characterization for Environmental Purposes With Emphasis on Soil, Rock, the Vadose Zone and Groundwater* (1996).

4.3.1 Survey and Preparation of Test Area. Site work begins with a cursory survey of the tentatively selected test area. Utility lines should have been located and marked. Potential obstructions such as trees, boulders, or infrastructure should be noted during this survey and compared to the tentative well locations within the proposed testing area. Although the final testing location will depend on the results of these characterization activities, unanticipated obstructions or hazards may exclude potential locations from further consideration and prompt the selection of alternative areas. Because sampling

locations need to be identified in the site-specific test plan, alternative locations should be considered during planning.

If no obstacles or hazards are detected, the plots are prepared for drilling or probe insertion. Preparation includes the following:

- Flag and clear selected locations to allow easy access for the drill rig.
- Establish site work zones in accordance with the HASP. At a minimum, delineate the exclusion zone and decontamination area.
- Make certain necessary permits and approvals have been obtained before proceeding with drilling, digging, or system component installation. A copy of necessary permits should be kept on site throughout the course of the project.

4.3.2 Analytical Methods. Before appropriate drilling and sampling methods can be selected, the analytical testing regimen needs to be outlined. The characterization of the potential testing location requires the analysis of both aquifer cores and groundwater. Samples undergo both field and laboratory analysis.

Aquifer cores are collected from at least 20% of the groundwater sampling locations during the installation of the test system. The soil type and stratigraphy is documented in the field based on visual observations of all cores. Subsamples are then taken from the cores and sent to an off-site laboratory for the analyses. Table 4.1 lists EPA accepted methods for all of the required analyses. These are standard methods that are performed by most contract analytical laboratories. Alternative methods can be used provided that their precision and accuracy has been demonstrated and that they are approved by the appropriate regulatory agency.

Table 4.1. Analytical Methods for Examination and Testing of Aquifer Cores.

Analysis	Method	Testing Location
Soil type	Direct visual examination	Field
Stratigraphy	Direct visual examination	Field
VOCs	SW 846 Method 8260B	Laboratory
TOC	SW 846 Method 9060	Laboratory
Total Iron	SW 846 Method 7380	Laboratory

In addition to aquifer core samples, groundwater samples must be taken and characterized. Each of these samples is analyzed in the field for dissolved oxygen, pH, temperature, ferrous iron content and conductivity. Samples are sent to an off-site analytical laboratory for analysis of the analytes listed in Table 4.2. The methods listed are standard methods that are accepted by EPA and are performed by

most contract analytical laboratories. Alternative methods can be used provided that their precision and accuracy has been demonstrated and that they are approved by the appropriate regulatory agency.

Table 4.2. Analytical Methods for Testing of Groundwater Samples.

Analysis	Method	Testing Location
DO	DO probe	Field
Temperature	Digital thermometer	Field
pH	pH probe	Field
Fe ⁺²	Hach test kit	Field
Conductivity	Conductivity meter	Field
Chloroethenes	SW 846 Method 8260B	Laboratory
DOC	EPA Method 415.1	Laboratory
NH ₃	EPA Method 350.2	Laboratory
CH ₄ , C ₂ H ₄ , C ₂ H ₆	SW 3810 modified or Kampbell et al., 1989	Laboratory
NO ₃ , NO ₂ , SO ₄	EPA Method 300	Laboratory
Cl, Br	EPA Method 300	Laboratory
Conductivity	EPA Method 120.1	Laboratory
Alkalinity	EPA Method 310.1	Laboratory
PH	EPA Method 150.1	Laboratory
Iron	EPA Method 3500-Fe	Laboratory

4.3.3 Aquifer Core Collection Methods. Aquifer cores are collected for porosity testing, delineating stratigraphy, and contaminant and geochemical parameter analysis. Several methods are commonly used to collect aquifer cores; and the selection of a specific method depends on the depth of sampling, the volume of sample required, and the method of drilling. The following subsections briefly describe three acceptable core sampling methods, the split-spoon, core-barrel, and direct-push methods.

4.3.3.1 Split-spoon sampling. One of the most common methods for collecting soil cores is to use a split-spoon sampler. Most often, this method is used in conjunction with hollow-stem auger drilling. This method has the advantage of leaving the auger flights in place during sampling, which significantly cuts the time of drilling and eliminates the potential for hole collapse. To collect a core sample, the hole is advanced to the desired depth, and then the spoon is driven into the undisturbed formation ahead of the bit using a percussion hammer. The method is good for collecting samples in cohesive sediments. Low-volume recovery can result when sampling coarse sands, especially below the water table. Sample retainers can be placed in the cutting shoe to help prevent slippage of the sample during spoon retrieval.

Split-spoon samplers consist of three sections: a cutting shoe, a sample chamber, and a drive cap. Spoons with diameters between 1 and 2 inches and sample chamber lengths of 1.5 to 2 feet are most commonly used to collect core samples. The sample chamber is split lengthwise into two halves with the edges machined so that the pieces fit together to form a tight seal. Threads are machined onto each end of the chamber to accept the cutting shoe and drive cap, which screw on and keep the two halves of the chamber together. The inside of the drive cap may contain a type of check valve to help retain materials in the chamber during sample retrieval.

4.3.3.2 Core-barrel sampling. Core-barrel sampling is another method for retrieving undisturbed soil cores. Core-barrel samplers provide larger samples than split-spoon samplers and are well suited for sampling clays and silty sands. Coarse sands are more difficult to retrieve.

Sample collection is similar to split-spoon sampling. Hollow-stem augers are advanced to the desired sampling depth, and then the core barrel is pushed into the formation ahead of the drill bit. Once the core barrel is advanced to the desired depth, it is retrieved from the hole and the sleeve liner containing the sample is removed. The ends of the sleeve liner are capped. The core is then examined for stratigraphy; the findings are recorded as a function of depth in a core logbook. The core is cut into sections and the ends of each section are then capped and sealed. The sections are then labeled, recorded in a field notebook, and prepared for shipping to the laboratory conducting the porosity, contaminant, or geochemical analyses.

Core barrels consist of a cutting edge, a single-piece sample chamber, and a drive cap. Typical configurations are available with diameters between 1 and 3 inches and lengths up to 4 feet. Sleeve liners are available but are not as common as with split-spoon samplers.

When the diameter of the core barrel is close to, or greater than, the internal diameter of the auger flights, the augers must be removed from the borehole for sample collection. This can cause a significant increase in drilling time.

4.3.3.3 Direct-push core sampling . Direct-push sampling methods are alternatives to the methods that require drilling. Use of direct-push methods should be given consideration when cores can be collected independent of system component installation and when sampling unconsolidated sediments at depths of up to 60 feet. The achievable depth is dependent on both the hydrogeology of the site and the

diameter of the sampling probe. As with the other sampling methods, recovery of coarser sands can be difficult. A soil retainer can be used to help prevent loss of the core material during retrieval.

Several vendors, including Geoprobe of Salinas, Kansas, and Arts Manufacturing & Supply of American Falls, Idaho, offer direct-push sampling systems, and many drilling contractors offer direct-push sampling services. Although the specifics of the systems offered by the different vendors may vary, their basic operating principles are similar. Core samples are collected by pushing a sampling probe into the subsurface using a combination of hydraulic push and pneumatic hammering.

Direct-push samplers are available in sizes ranging from 1 to 2 inches in diameter and up to 4-foot lengths. Metal and plastic sleeve liners are used to collect the core. Plastic sleeves allow for direct observation of core stratigraphy, but are not as resistant to deformation during probe advancement.

Direct-push samplers come equipped with releasable piston drive points that facilitate sampling a discrete depth interval without requiring continuous coring from the surface. When using samplers that require continuous coring, care must be used to prevent sloughing of wall materials into the hole during insertion of the sampler back into the borehole.

A distinct advantage of direct-push sampling is that it does not produce drill cuttings which often require special handling and disposal procedures. Two disadvantages are that the diameter, and hence the volume of core, and the depth of sampling are limited.

4.3.4 Groundwater Sampling. During characterization, groundwater samples are collected for analysis of contaminants and other relevant geochemical parameters to define both the horizontal and vertical extent of these parameters. Samples may be collected using any of a number of methods, including direct-push and monitoring well sampling. Because of the volatile nature of many of the analytes of interest, the sampling method must minimize contact between the sample and the atmosphere. Exposing the samples to air during collection can cause loss of contaminant and can oxygenate the sample, interfering with dissolved oxygen and/or oxidation-reduction potential (ORP) measurements.

ASTM Standards D 6001, *Direct-Push Water Sampling for Geoenvironmental Investigations* (1997) and D 4448, *Standard Guide for Sampling Groundwater Monitoring Wells* (1986) provide an excellent source of detailed information about groundwater sampling procedures. In addition, the USEPA has

published detailed sampling information in its *Handbook for Sampling and Sample Preservation of Water and Wastewater* (EPA-600/4-82-029, 1982).

4.3.5 Aquifer Testing. The success of any size RABITT system depends upon the ability to distribute amendments through the groundwater, and therefore, aquifer properties affecting amendment distribution need to be characterized. Primary measurements of aquifer characteristics include hydraulic conductivity, water table elevation, and effective porosity. These three characteristics can be used to determine the groundwater flow direction and velocity. The groundwater flow direction is important, because placing the test system parallel to the direction of flow will maximize the communication between the injection wells, the monitoring wells, and the extraction well. Although it is possible to manipulate the groundwater flow direction by pumping from the extraction well, proper orientation of the test system will minimize the need for pumping and subsequent treatment of contaminated water. The groundwater velocity is important because it will dictate the hydraulic residence time in the testing zone and thus dictate the spacing of the below-grade system components.

The following aquifer testing approach was developed to obtain the information required to determine both the groundwater flow direction and the groundwater velocity, while minimizing the costs associated with sophisticated pumping tests:

1. Measure the hydraulic gradient and develop flow nets to map the groundwater flow direction.
2. Drill a borehole for installation of a well that will be used as an injection well in the treatability test system.
3. Core the aquifer during drilling to log stratigraphy, to provide samples for contaminant and geochemical analyses, and to measure porosity.
4. Install a well into the borehole so that the screened interval is placed in a contaminated stratum of sufficient permeability.
5. Conduct slug tests to determine the hydraulic conductivity.
6. Calculate the groundwater velocity using Darcy's law.

The following subsections provide detail on the methods used to measure the parameters in this aquifer testing procedure.

4.3.5.1 Groundwater flow direction. The direction of groundwater flow can be determined by measuring the static water table elevations in existing monitoring wells around the site. To get the net direction of groundwater flow, the monitoring wells must be installed with screened sections that penetrate the aquifer. Water levels in partially penetrating wells may provide erroneous results. A more precise method for determining the direction of groundwater flow would be to measure the hydraulic head in wells screened across the stratigraphic layer where the test system will be installed. However, it is very unlikely that a well meeting this criterion will be available. It may be desirable to check the flow direction using water table elevation measurements from the wells installed during this investigation to better orient the test system. At least three water-level measurements are required to determine a flow direction; however, it is important to remember that more measurements will yield a more accurate analysis.

Water table elevations are most easily measured using an electronic water-level indicator. These indicators consist of a probe in which an electrical circuit is completed when the probe contacts water. The probe is connected to a wire or line that is marked with depth graduations, usually in 0.01-foot increments. The probe is lowered down the well, and when it encounters the water table the circuit is completed and a signal is observed at the ground surface. The depth measurement is read at the top of the well casing and the water table elevation is calculated by subtracting the recorded measurement from the surveyed elevation of the top of the well casing. This procedure is repeated in as many wells as possible to provide a sufficient database for delineating the groundwater flow direction.

The water table elevation data are used to develop a flow net, which is a graphical representation of the flow field consisting of equipotential lines of hydraulic head and flow lines. Flow nets are constructed by plotting the water table elevations at their respective longitude and latitude coordinates on a map of the site and determining equipotential lines of hydraulic head. In homogeneous, isotropic aquifers, groundwater flows perpendicular to the equipotential lines in the direction of decreasing gradient. In anisotropic aquifers, the flow lines are not perpendicular to the equipotential head lines (Fetter, 1994).

4.3.5.2 Effective porosity. Porosity is defined as the percentage of the total volume of a soil that is void of solid material (Fetter, 1994). A more useful term for determining groundwater flow velocities is the effective porosity (η_e), which is defined as the percentage of interconnected pore space (Domenico and Schwartz, 1990). The following procedure for determining η_e is based on the method described by Fetter (1994).

1. Cut out a 4-inch section of undisturbed core material collected during the installation of the well described above. Keep the core intact in the sleeve liner. Measure the volume of the core material and the volume of the sleeve liner.
2. Dry the core in an oven at 105°C; cool and weigh. Repeat this procedure until a constant weight is achieved.
3. Fill a glass jar with a screw-cap lid with distilled water to the level that will allow complete submergence of the 4-inch core. Make sure to leave enough room above the water level to allow the water to rise when the core is submerged without overflowing. Place the jar on a level surface and mark the water level.
4. Slowly submerge the core into the water, taking care to prevent core material from coming out of the sleeve.
5. Cap the jar, and place on a level surface.
6. Observe the water level in the jar. When the water level equilibrates, the core is saturated. Mark the water level on the jar.
7. Remove the core from the jar.
8. Fill the jar to the original water level, then add a measured amount of water to bring the level to the water level marked when the core was saturated.
9. Calculate η_e by subtracting the volume of the sleeve liner from the volume of water required to raise the water level, then dividing the result by the volume of core material.

4.3.5.3 Hydraulic conductivity. Now that the hydraulic gradient and porosity are known, the only remaining parameter that must be measured to calculate the groundwater velocity is the hydraulic conductivity. The hydraulic conductivity is a coefficient of proportionality that describes the rate at which water can move through a formation (Fetter, 1994). Hydraulic conductivity can be measured using any of a number of techniques. The most appropriate method for conducting the type of treatability test described in this protocol is the slug test. Slug testing is described in detail in ASTM D 4044 *Test Method (Field Procedure) for Instantaneous Change in Head (Slug Tests) for Determining Hydraulic Properties of Aquifers* (1997). Two of the primary advantages to using the slug test method are that it involves a simple field test and that it produces little or no contaminated water that requires treatment or disposal.

Slug tests can be conducted by either increasing or decreasing the water level in a monitoring well. The water level is rapidly changed by adding a solid "slug" of known volume to the well, after which recovery to pretest levels is measured over time. The procedure described below increases the water level without removing contaminated groundwater. Because the well will be screened in permeable formations, the recovery of the water level may be rapid. Consequently, a pressure transducer with an automated data logger should be used to monitor head changes.

The following procedure is followed for conducting the slug test.

1. Install a pressure transducer into the well at some depth below the water surface to minimize potential interference in depth measurements due to turbulence during water introduction. Record the static water level in the well in which the slug test is to be conducted.
2. Select a "slug" of sufficient volume to raise the water level at least 3 to 4 feet (for a ½-inch-diameter well this volume will be approximately 1 pint).
3. Set up the data logger to record the water level every 0.5 second.
4. Rapidly drop the "slug" into the well.
5. Wait for the water level to return to pretest levels.
6. Repeat steps 3 through 5 two times so that the test is run a total of three times.

Because the test is conducted in a monitoring well that does not completely penetrate the aquifer, the data generated from the three tests are analyzed to determine a value for hydraulic conductivity by the Hvorslev method, using the following equation (Hvorslev, 1951).

$$K = \frac{r^2 \ln(L_e/R)}{2LT_0} \quad (5)$$

Where: K = hydraulic conductivity (L/T)
r = radius of well casing (L)
L_e = length of the packed interval around the well screen (L)
R = radius of the borehole (L)
T₀ = time required for water level to fall 37% of initial change (T)

The value for T_0 is determined graphically by plotting the ratio of the water level as a function of time against the water level immediately following addition of the slug. The data are plotted on semilog paper with the head ratio on the log scale. The plotted data should be linear. T_0 is defined as the time required for the head ratio to equal 0.37.

4.3.5.4 Groundwater velocity. After the hydraulic conductivity has been determined, Darcy's law can be applied to determine the groundwater velocity as follows.

$$v = \frac{-K}{\eta_e} \frac{dh}{dL} \quad (6)$$

Where:

v	=	Darcy velocity (L/T)
K	=	hydraulic conductivity (L/T)
dh/dL	=	hydraulic gradient (L/L)
η_e	=	effective porosity

4.4 Refinement of Conceptual Model. The data obtained from aquifer testing and the analysis of soil and groundwater samples should be incorporated into the previously constructed conceptual model. The model can now include a localized vertical profile of the stratigraphy, hydraulic conductivity, and contaminant concentrations for the potential testing area. This information should be compared to previous data and extrapolated to surrounding areas. If all goes well, the new data will corroborate the existing data that led to the selection of the potential testing location, and previously constructed data contours can be updated to reflect the recently collected data. Any discrepancies that exist between new and existing data should be resolved before continuing. This may require a second round of sampling in the potential testing location or from other locations around the plume. It is vital that the conditions within the testing area are known with confidence before initiating the treatability test.

4.5 Assessment of Potential Testing Area. The data collected from aquifer cores, groundwater samples, and aquifer testing should provide all the information necessary to evaluate the potential testing area for RABITT application. This data can be used in conjunction with the rating system outlined in Section 3.4 to assess the probability of stimulating biologically catalyzed reductive dechlorination within the potential testing area. Because the rating system omits less quantifiable factors, the following need to be considered independently:

- Is the potential testing location representative of the site in general?
- Is the contaminant concentration high enough so statistically significant decreases can be measured?
- Are the groundwater flow direction and velocity predictable and favorable?
- Will the subsurface stratigraphy impede or channel flow through the test zone?

After assessing the candidate testing location's potential, one of the following four decisions needs to be made based on all available site data:

1. Promising testing areas that are technically feasible and meet project goals can be approved for treatability testing.
2. Potentially promising testing locations with data discrepancies can undergo a second round of sampling to resolve the discrepancies.
3. A dubious testing location, due to some highly localized feature, may be abandoned and another potential testing location selected for characterization.
4. The site as a whole may be determined unfit for RABITT if characterization of the potential testing location demonstrates a previously undetected but potentially site-wide limiting condition.

Regardless of the final decision, the project manager and facility POC should be contacted to discuss the next step in the process. If the testing location is technically feasible and meets project goals, final approval of the testing location should be obtained from the project manager and preparations for microcosm testing should begin. If additional sampling will be required, an additional work plan will need to be prepared and submitted. Characterization of another testing location will require each step in Section 4.0 be repeated until a testing area is selected or the technology is dropped from further consideration. If the technology is to be dropped from further consideration the reasons should be outlined and presented to all concerned parties.

5.0 MICROCOSM STUDY

5.1 Importance of Site-Specific Investigations

Microbial systems differ in terms of the fermentation pathways used to degrade the primary substrates that might be chosen for enhancing reductive dechlorination. For example, lactate or ethanol are normally expected to be fermented rather rapidly to acetate and H_2 , resulting in high H_2 levels that persist for only short periods as various H_2 -using organisms deplete it. However, in some environments, lactate or ethanol may be fermented to propionate, which itself can serve as a more slowly fermentable source of persistent, low H_2 levels, thus making lactate or ethanol (normally poor choices for enhancement) good choices at some sites.

Such site-specific differences in the fate of the supplied donor underscore the importance of conducting proper microcosm studies in advance of, or to provide parallel support for, field-scale studies of enhancement options. All too often, investigators and practitioners take a "black-box" approach to enhancement, wherein primary substrates are added without proper determination of their fate. In such cases, no electron balances are performed to track the proportion of substrate channeled to dechlorination vs. competing processes. This oversight can lead to bewilderment over why ethanol worked at site A but not at site B.

Another issue affecting the success of enhanced remediation is nutrition. Dechlorinating microorganisms may be dependent upon other organisms in the environment for necessary growth factors (e.g., vitamins, essential fatty acids, etc.). The choice one makes of added electron donor (e.g., ethanol vs. lactate) selects the population of nondechlorinators, and therefore affects the resulting production and level of these microbially produced growth factors in the diverse culture.

Finally, we know little of the diversity of organisms capable of dechlorination. In recent years several bacterial cultures have been isolated that are capable of dechlorinating PCE as far as *cis*-DCE; only one bacterium thus far has been isolated that is capable of completely dechlorinating PCE or TCE to ethene. However, many sites exhibit complete dechlorination; many more exhibit VC accumulation, and still more exhibit *cis*-DCE accumulation. These site-to-site differences in the extent of dechlorination may reflect differences in microbial composition (i.e., true differences in microbial potential). However, other limiting factors may be involved, such as a lack of sufficient electron donor or nutrients, or unfavorable environmental parameters.

Site-specific differences in native microbial populations and the environment necessitate site-specific microcosm studies to evaluate alternative enhancement strategies.

5.2 Sample Collection

Preparation of microcosms requires that two types of samples be obtained from the testing zone, i.e., subsurface soil material and adjacent groundwater. In both cases, care should be taken to minimize exposure to atmospheric oxygen.

5.2.1 Soil Samples. Soil sampling methods have been described earlier (see Section 4.3.3), but regardless of the soil collection method used, care should be taken to avoid exposing the cores to air or microbial contamination when they are being prepared for storage and shipment. Microcosm studies using soil cores from the site are designed to mimic the in situ microbial ecology as closely as possible; and exposure to air has the potential to change the predominant microbial consortium within the sampled material.

When a coring device is used to generate a core sample for use in an anaerobic study, sleeve liners are placed into the sample chamber. Brass or stainless steel sleeve liners are available. As the spoon is driven, the soil enters the sample chamber through the cutting shoe and is retained in the sleeve liners. Once the spoon has been driven to the desired depth, it is retrieved and split open, and the sleeve liners are removed. Immediately after removing the sleeved cores from the collection spoon, the sleeve ends should be covered completely with Teflon™ sheets and capped. The caps should be positioned in such a way that the Teflon™ sheeting is not wrinkled and an airtight seal is provided on the sleeve ends. The caps should then be taped securely to the sleeve to maintain the airtight seal. The sleeve should be labeled with a permanent marker or paint pen with the sample identification, collection location, depth of collection, time and date, and orientation (that is, there should be an indication of which end of the sleeve was deeper in the soil column). The capped sleeve should then be placed in a sealable plastic bag and placed in a cooler with frozen gel packs.

All of the collection information that was written on the sleeve label should be copied to the bound field logbook. Any appropriate comments or observations made during the collection and sealing process should also be recorded in the logbook and associated with the sample record entry. Shipment of collected samples should occur as soon as it is feasible (i.e., when one cooler is full, it is shipped).

A convenient, inexpensive means of storing and transporting unconsolidated soil samples (e.g., from hand-augering) is the use of standard, 1-qt canning jars (e.g., Mason™, Ball™, or Kerr™). They are first filled completely with groundwater from the site. Sediment is added to the water-filled jars directly from the sediment-sampling device, causing displaced groundwater to overflow, but allowing minimal contact of soil with air. When the solid material has nearly filled the jar, the lip is wiped clean of any grit, the jar is topped completely with groundwater (in fact, a meniscus can be achieved above the rim), the lid is affixed, and the retaining ring is threaded into place. Using this technique, it is possible to obtain a sample with virtually no visible gas bubbles.

5.2.2 Groundwater Samples. Groundwater samples should be obtained that are as representative as possible of the water in contact with the soil samples described in the preceding subsection. This often means obtaining groundwater from sampling wells adjacent to the site from which soil samples have been taken — and at the same level. Groundwater sampling is described in Section 4.3.4. Samples should be kept in a cooler, if possible, until used. In any event, care should be taken to prevent exposure to temperatures above 35°C. No preservatives should be employed with groundwater samples intended for microcosm preparation.

5.3 Run Studies

5.3.1 Microcosm Preparation. Microcosms are prepared in 160-mL serum bottles, with Teflon™ lined, butyl-rubber septa (Wheaton 224100-175, autoclaved before use to drive off organics that potentially could interfere with the analysis) and aluminum crimp caps. A mixture of subsurface soil (50 g dry wt)¹ and groundwater from the site (50 mL) is recommended, obtained as described in Sections 4.3.3, 4.3.4, and 5.2.

If groundwater analyses indicate bicarbonate alkalinity < 0.05 eq/L, NaHCO₃ buffer should be added to the microcosms to achieve that level in aqueous phase. It is also recommended that groundwater for

¹ The soil, of course, is not added “dry.” We suggest that three representative samples (later discarded) be analyzed for moisture content, establishing the ratio of wet weight to dry weight for the soil material. From this, a mass of wet soil equal to 50 g dry weight can be distributed to each microcosm bottle. Although this may seem excessively meticulous, it provides knowledge of the true content of water in the microcosms and thus is potentially useful for later data interpretation.

microcosm use be amended with resazurin (< 1 mg/L to avoid toxicity), as an indicator of low redox potential.²

Microcosms should be prepared in an anaerobic glovebox under a 1 to 3% H₂ (balance, N₂) atmosphere. After preparation, sealed microcosm bottles are removed from the glovebox and purged 10 minutes on the benchtop with a cannula, using O₂-free anoxic gas (30%CO₂/70%N₂), scrubbed of O₂ by passing through 350°C copper catalyst (Gerhardt et al., 1994) or reduced titanium solution (Zehnder and Wuhrmann, 1976), and then resealed. Standard anaerobic techniques should be employed, taking care not to introduce air (Gerhardt et al., 1994). During benchtop purging operations, it is suggested that a few representative bottles be tested for pH, using a thin probe that can be inserted directly into the serum bottle. The pH should be between 6 and 8. If not, the pH can be adjusted by raising or lowering the CO₂ content of the purge gas.³

After purging and resealing the microcosm bottles, the preparer should add 5 to 10 mL of the anoxic purge gas, via syringe, to overpressure the bottles as insurance against the introduction of air during subsequent sampling events. It is a good idea to calculate, *a priori*, the expected gas production from anticipated microbial transformations, and to include this consideration in the choice of overpressuring volume. The goal is to maintain an overpressure at all times, but not more than 0.5 atm of overpressure (or else significant loss of analytes may occur during sampling events). This consideration is further addressed in Section 5.3.2, where analytical procedures are discussed.

Triplicate microcosms should be prepared from anoxic 100- or 1,000-fold concentrated stock solutions. The solutions should be added using syringes that have been flushed with N₂ or other inert gas, and fitted with 25-gauge × ½-inch needles. Triplicate microcosms should be prepared for each condition shown in Table 5.1.

² Resazurin is colorless at E_H < -110 mV and pink/purple at higher values (Gerhardt et al., 1994). This E_H of color change is not sufficiently low that colorlessness guarantees that adequate reducing conditions have been maintained for the most stringent anaerobes. In other words, from mishandling, or poor anaerobic technique, conditions can become too oxidizing for some dechlorinators without resazurin's tell-tale pink color appearing in evidence; however, later development of color provides indication, *post-mortem*, that failure resulted from excessively oxidizing conditions.

³ Although the pH of the native soil/groundwater may be decidedly non-neutral, this protocol suggests the use of neutral conditions for conducting microcosm studies to maximize the likelihood of a successful result, recognizing that similar buffering of the in situ treatment zone may be required. The addition of a donor, its fermentation to intermediate volatile fatty acids, and the reductive dechlorination process itself are all processes with potentially significant impacts on alkalinity and pH. Where high concentrations of donor and/or chloroethenes are involved, buffering becomes a necessary fact of life in the deployment of RABITT.

Table 5.1. Conditions to be Examined in Serum Bottle Microcosm Studies.

Bottle Set	Donor	Yeast Extract Addition (20 mg/L)	Vitamin B₁₂ Addition (0.05 mg/L)
1	None (Autoclaved, Abiotic Control)	No	No
2	None (Biotic Control)	No	No
3	None	Yes	Yes
4	Yeast Extract (200 mg/L)	No	Yes
5 (A)	Lactate (3 mM)	No	No
5 (B)	"	Yes	No
5 (C)	"	No	Yes
5 (D)	"	Yes	Yes
6	Butyrate (3 mM)	Yes	Yes
7	Lactate/Benzoate Mixture (1.5 mM each)	Yes	Yes

Depending on the levels of chloroethenes already present, PCE/TCE may or may not be administered. Ideally, initial PCE or TCE levels should be around 30 μM (corresponding to 5 ppm PCE) in microcosm bottles, which is high enough for analytical convenience but low enough to avoid toxicity. Some of these decisions must, necessarily, be site-specific.

The autoclaved, abiotic controls (Bottle Set 1) that accompany the live microcosms should be autoclaved twice on successive days before adding (or restoring) PCE or TCE. These controls serve to provide estimates of abiotic losses (e.g., losses through the septum) of PCE or TCE from the bottles over the test period.

The biotic controls (Bottle Set 2) to which nothing (no vitamins, buffers, or YE, except possibly PCE/TCE, if deficient) has been added will be useful for assessing the background microbial activity that occurs in the bottles in the absence of additional microbial activity stimulated by the amendments.

The bottles receiving no donor but low-level yeast extract and vitamins (Bottle Set 3) will demonstrate whether native organisms are limited only by lack of nutrients.

Bottles receiving the high-level (200 mg/L) yeast extract amendment (Bottle Set 4) and vitamins serve to screen for possible stimulation of dechlorination by donors that we are not able to test individually. Yeast extract consists of a mixture of many different types of donors, and still others are produced upon its fermentation. These bottles will show whether dechlorination is possible through amendment with donors other than the specific ones selected for testing (i.e., lactate, butyrate, and benzoate).

Lactate has been reported to be a successful donor for stimulating dechlorination in a number of studies. It is often fermented to propionate. Since it is a likely successful donor, it will be tested as a donor under four different conditions (Bottle Sets 5A-5D) of trace nutrient amendment to determine first whether lactate is a successful donor, and second whether the addition of low-level yeast extract and vitamin B₁₂ is actually necessary for successful stimulation of dechlorination activity.

Butyrate-amended bottles (Bottle Set 6) and a lactate/benzoate mixture (Bottle Set 7) will also be tested to assess the success of slowly-fermented, low-level-hydrogen-generating donors.

The above-described protocol requires incubation of 30 bottles per site location to be subjected to the microcosm study.

5.3.2 Incubation and Analyses. Microcosms are incubated at ambient laboratory temperatures (20-25°C) under quiescent conditions (Note: Agitation is preferred, but probably impractical due to the large numbers of bottles employed). Though such temperatures are likely higher than subsurface field temperatures, higher temperatures should accelerate the microcosm studies, without seriously altering the relative results of electron-donor comparisons.

Bottles should be routinely monitored (initially once per week, but less often where weekly analyses suggest so) for remaining, supplied electron donor, chloroethenes, volatile fatty acids, methane, H₂, and (where appropriate) toluene, which can be a significant source of reducing equivalents via its fermentation at sites containing BTEX co-contaminants.

When analyses indicate depletion of PCE/TCE and/or electron donor, these constituents should be restored to their original levels. Incubation should be continued for a total duration of six months or three PCE/TCE depletion cycles, whichever occurs first.

5.3.2.1 Determination of volatile organic compounds by gas chromatographic analysis of headspace samples. Section 4.3.2 outlines analytical methods for groundwater monitoring; however, due to the generally large sample sizes required, these methods are inappropriate for the microcosm study. This section outlines appropriate analytical methods for monitoring the course of the microcosm study.

Volatiles (H₂, CH₄, chloroethenes) can be measured conveniently using headspace samples (0.1 to 0.5 mL) with gas chromatography (GC). Headspace sampling should be performed with a locking, gastight

syringe. The goal is to sample the microcosm's headspace at native temperature and pressure; consequently, the syringe should be locked before extraction of the needle from the microcosm serum bottle and only unlocked again when the sample is injected into the GC. Careful accounting should be made of the total gaseous volume sampled over time; it is important to avoid creating a vacuum in the microcosms because air can be drawn in during sampling events, ruining the microcosm study. When accounting procedures (i.e., consideration of cumulative gaseous and liquid samples removed, mitigated by microbially produced gases) suggest there is a danger of creating a vacuum, the operator should add (via syringe) additional anoxic gas volume to microcosms.

H₂ at lower levels (< 250 to 2,000 nmol/bottle) will require the use of a reduction gas detector (RGD) (Trace Analytical, Inc. Menlo Park, CA), whereas higher levels can be quantified with a thermal conductivity detector (TCD).⁴ Use of the TCD for H₂ measurement requires N₂ as the GC carrier gas (rather than He), because the thermal conductivities of H₂ and He are too similar to achieve the needed sensitivity in H₂ detection. Because the TCD is "nondestructive" of the sample, operation of a TCD in series with an RGD allows a wide range of application. With most microcosms, the bulk of H₂ measurements will require the RGD. CH₄, and ethene are quantified with a flame ionization detector (FID).

Chloroethenes at aqueous concentrations > 5 ppb generally can be measured with the FID, but if there are co-eluting, nonchlorinated compounds causing interference with the chloroethenes, analysts may find it convenient to use an electron capture detector (ECD) because the nonchlorinated compounds will be "invisible" to it. The ECD also is a "nondestructive" detector. Thus, it is possible to operate an ECD in series with (followed by) an FID. However, when doing so, analysts cannot employ CH₄/Ar as the carrier or makeup gas; the use of N₂ causes some loss of sensitivity with most ECD designs, but this should not be a critical concern in most instances.

A number of different GC columns, packed or capillary, are suitable for the above-described headspace analytical procedures. One complete system that accomplishes the entire suite of analyses from a single headspace injection, as described by Fennell (1998), utilized the following procedure:

Analysis of PCE, TCE, DCEs, VC, ETH, CH₄ and H₂ was performed with two GCs equipped with two FIDs, one TCD, and a stand-alone RGD (the latter two in series). A single 0.1- or 0.5-mL headspace sample removed from the reactor or serum bottle headspace via a locking gas-tight syringe was injected into the system. Two columns were used to separate components and

⁴ The useful range of each detector is instrument- and sample-size specific; the useful range for H₂ measurement by RGD is between about 5 to 2,000 nmol/bottle, based on a 0.1-mL sample.

two air-actuated four-port switching valves were used to direct the carrier gas streams and the components to be detected to one of the three different detector types. The first column in series was a 1/8-inch diameter, 8-ft stainless-steel column packed with 1% SP-1000 on 60/80 Carbopack-B (Supelco, Inc.). The second column was a 1/8-inch diameter, 10-ft stainless-steel column packed with 100/120 Carbosieve-G (Supelco, Inc.). Both columns were contained in the oven of GC #1 and were subjected to the same temperature program. N₂ gas (ultra high purity, 99.998 %, Matheson Gas Co.), at 30 to 35 mL/min was the carrier flow. Prior to passing into the GC system, the carrier was first passed through a catalytic combustion filter (Trace Analytical) to remove the RGD contaminants CO and H₂ and through a molecular sieve (Supelco, Inc.) to remove water and hydrocarbons. The FIDs were maintained with H₂ and air. The TCD was maintained with N₂ carrier and reference gas flows (ultra high purity, 99.998 %, Matheson Gas Co.), at 30 to 35 mL/min. The outputs from these detectors were integrated by their respective GC integration systems.

When a sample was injected, the GC system was activated and relays programmed to actuate the switching valves at specific times controlled to which detector the separated compounds were directed. The oven temperature was maintained at 90°C for the first 2.8 min and was then ramped to 200°C at 30°C per min. The temperature was held at 200°C for an additional 9.1 min. The injector temperature was 200°C and the detector temperature was 250°C. The main carrier gas flow was directed through the two columns to the TCD in GC #2 and the RGD for the first 1.38 min, while H₂ passed rapidly through the columns and entered the TCD and then the RGD, in that order. After 1.38 minutes, Valve 2 switched positions and the main carrier gas flow was then connected to FID 2 and auxiliary flow 2 was connected to the RGD. CH₄ and ETH passed relatively quickly through the Carbopack column and entered the Carbosieve where they were separated and detected by FID 2. After 1.4 minutes Valve 1 changed positions and the main carrier gas flow passed through the Carbopack column which separated the chloroethenes PCE, TCE, and VC. The DCE isomers came out together on this column. PCE, TCE, and VC were eluted from the Carbopack column to FID 1. Auxiliary flow 1 flowed through the Carbosieve column and continued to elute CH₄ and VC to FID 2. Over the time period of this study, flow rates and programming times changed somewhat, however, typical retention times of all the compounds are shown in Table 6.2. (pp. 87-94).

Table 5.2. Retention Times for Compounds from Single-Injection Gas Chromatography Analysis

Compound	Retention Time (min)
PCE	14.5
TCE	8.9
DCE (all isomers)	6.0
VC	2.3
ETH	8.4
CH ₄	3.1
H ₂	1.1

Standards should be prepared by adding known masses of analytes to microcosms that have been previously autoclaved and purged of VOCs (and analyzed after purging to demonstrate the absence of analytes). When calibrating the RGD at low H₂ levels, the purge gas must be specially purified (catalytic combustion filter, Trace Analytical, Inc.) to remove traces of H₂. In some microcosm

environments, it will be impossible to achieve H₂ levels below RGD detection limits by purging alone. In such cases, reasonable calibrations for H₂ (also for CH₄) may be achieved in previously purged bottles containing water with the same headspace volume as in the real microcosms. The high Henry's constant for H₂ (or CH₄) causes so little of its total inventory to be in the aqueous phase, and it is nonsorbing, that little error results from the use of such artificial conditions for H₂ (or CH₄) calibration. However, for other analytes (particularly those that may sorb to soil material), calibration requires the addition to bottles of known masses representative of microcosm conditions. Standards should be prepared as follows:

1. Autoclave twice (on two subsequent days).
2. Purge VOCs using sterile anoxic gases.
3. Allow to re-equilibrate for 2 hours under agitation.
4. Analyze to ascertain they are free of volatile analytes.
5. Spike with standard amounts of the analytes (see Gossett, 1987).
6. Equilibrate > 6 hours under agitation.
7. Analyze.

5.3.2.2 Analysis of volatile fatty acids. Volatile fatty acids (VFAs) can be measured by aqueous injection to a GC, using an FID for detection. Several columns are suitable; one example is the system described by Fennell (1998):

A GC with a 0.53-mm Nukol[®] × 15-m capillary column (Supelco, Inc.) and a FID was used for analysis of ethanol and VFAs. The N₂ carrier gas flow rate was 10 mL/min, the injector temperature was 200°C and the detector temperature was 250°C. For VFA analysis, a 0.5 µL sample was injected onto the column which was held at 90°C for 8 min, then ramped at 25°C/min to 110°C, and held for an additional 3 min. The retention times for these conditions were: acetic acid, 2.9 min; propionic acid, 4.5 min; isobutyric acid, 5 min; butyric acid, 7 min; isovaleric acid, 8 min; valeric acid, 9 min and hexanoic acid, 11 min.

A glass injector liner was used in the injector, and the Nukol[®] column was connected to a deactivated 5-m guard column at its ends between the column and injector and column and detector. It was important to change the septum and replace the liner with a clean liner every 50 to 60 VFA injections to rid the system of accumulated buildup of contaminants. A loop of the guard column was also removed periodically. (pp. 94-96)

Samples (0.5 mL) should be filter-sterilized (with 0.2-µm syringe filters) immediately when removed from the microcosm, and stored (refrigerated) in sealed vials with 10 µL of 8N H₃PO₄ per 0.5-mL sample. They are stable indefinitely under such conditions.

Accounting should be made of the cumulative liquid sample volume removed from the microcosms. When the sampled volume exceeds 5 mL, additional anoxic groundwater should be added (via syringe) to restore the microcosm liquid volume.

5.3.2.3 Analysis of lactate and benzoate. Lactate and benzoate can be measured by high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection. Fennell (1998) describes one such system for lactate analysis:

Lactic acid was analyzed by HPLC with either a 300-mm x 7.8-mm HPX-87H ion-exclusion column operated at 65°C, or a 100-mm by 7.8-mm Fast-Acid column operated at ambient temperature (Bio-Rad Laboratories), and a diode-array UV detector at 210 nm. The mobile phase was 0.013 N H₂SO₄, at 0.65 mL/min for the HPX-87H column and 0.7 mL/min for the Fast-Acid column. Samples of 0.5 mL were removed from serum bottles via a 1-mL syringe with a luerlock tip. They were filtered through a 0.2- or 0.45-μm PTFE filter (Gelman Sciences) into an HPLC vial, preserved with 10 μL of 6 N H₂SO₄, and capped with a septum and crimp cap and refrigerated. The HPLC injection volume was either 60 or 100 μL. (pp. 97)

Benzoate may be resolved with the Fast-Acid column as described above, using essentially the same method. The mobile phase in this case is recommended to be a mixture of 85% 0.01 N H₂SO₄ and 15% acetonitrile; detection is at 233 nm.

Again, accounting should be made of the cumulative liquid sample volume removed from the microcosms. When the sampled volume exceeds 5 mL, additional anoxic groundwater should be added (via syringe) to restore the microcosm liquid volume.

5.3.3 Additionally Recommended Microbiological Assessment (Optional). Site samples may be examined for the presence and numbers of various microbial populations, using most probable number (MPN) assays. Of interest in site assessment would be anaerobic heterotrophs; sulfate-reducers; H₂-using methanogens; acetate-using methanogens; H₂-using PCE/TCE dechlorinators; and YE-using PCE/TCE dechlorinators. Detailed procedures for these MPN assays are described by Maymó-Gatell et al. (1995). Although viable counts often underestimate the number of organisms present in an environment, they can provide a minimum number of the various physiological groups present. These numbers can be compared to the chemical measurements at the site and are useful in determining the physiological state of the organisms at the site (methanogenic, sulfate-reducing, etc.), and they provide some measure of the overall microbial activity. Moreover, high dilutions can provide source material for isolating organisms present at the site in high numbers, including dechlorinators, which may be different from those arising in enrichment studies. In the example from Table 5.3, it is clear that sulfate-reducing bacteria are among the predominant populations in the particular groundwater sample analyzed. The inability to detect PCE dechlorinators was consistent with the very low level of dechlorinating activity found in this particular sample.

Table 5.3. Most-Probable Number Analysis for Microbial Populations in a Groundwater Sample from NAS Fallon, Nevada.

Group	MPN/mL	Predominant Morphotype in Highest Dilutions
Anaerobic heterotrophs	4.3×10^5	Small rods
Sulfate-reducing bacteria	4.3×10^5	Small cocci
Methanol-utilizing methanogens (acetogens)	4.3×10^2	Large gas vesicle-containing packets resembling <i>Methanosarcina</i> .
H ₂ /CO ₂ -utilizing methanogens (acetogens)	4.3×10^1	Thick rods resembling <i>Methanobacterium</i>
PCE dechlorinators (H ₂ or YE as electron donor)	0.3×10^1	-----

5.4 Data Analysis to Determine Optimum Injection Formulation

The microcosm studies can provide valuable information concerning the fate of added reducing equivalents, including the pathways of fermentation operable at a site, and the potential competition for reducing equivalents among various microbial groups. As an example, Figure 5.1 depicts results from a microcosm prepared using subsurface material from Naval Air Station (NAS) Fallon, Nevada. Lactate was administered twice (day 0 and day 80), and though it was rather rapidly depleted, the lactate was significantly converted to persistent propionate at this site, and to acetate. This particular subsurface material was known to contain great quantities of sulfate. Estimating sulfate-reducing potential is difficult from chemical analyses alone, because the bioavailability of the sulfate is difficult to estimate. However, the microcosm results allow sulfate-reduction potential to be reasonably inferred from good electron-equivalents balances.

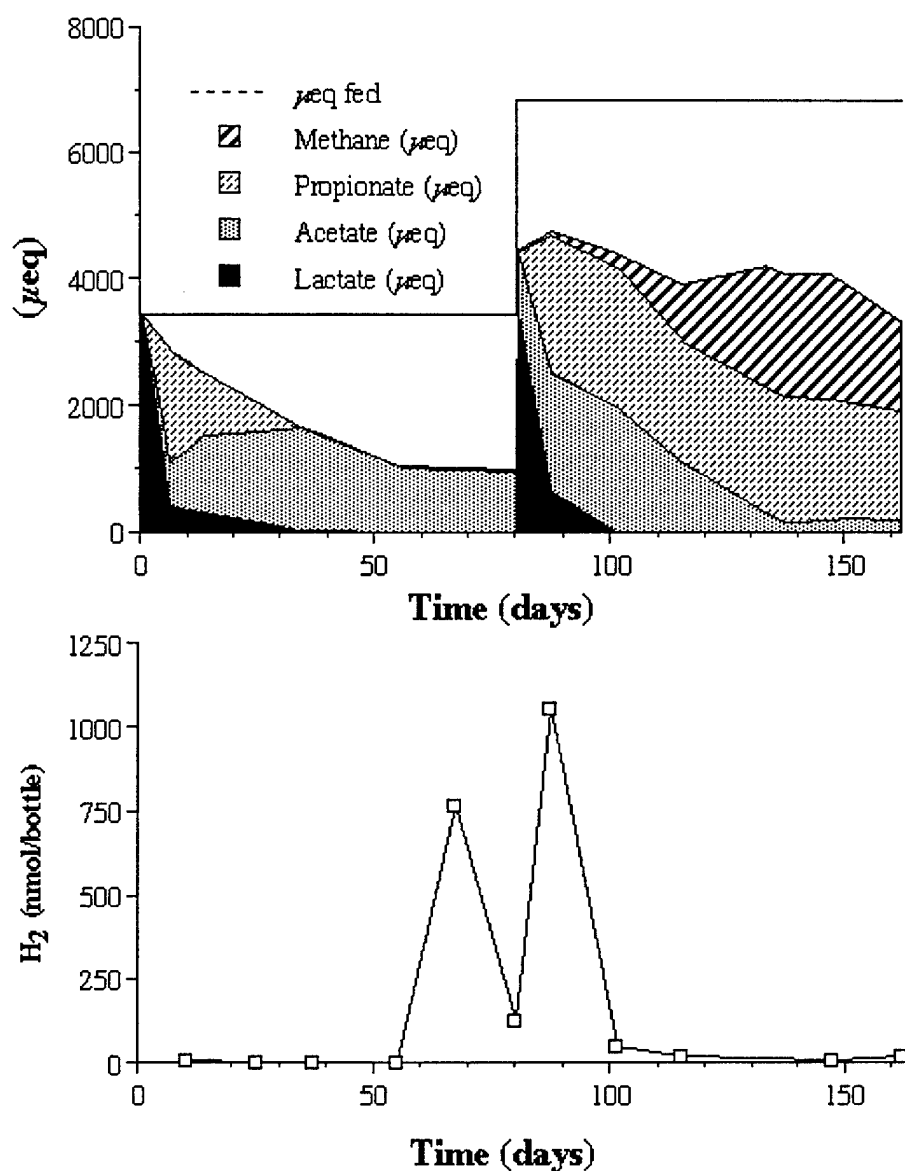


Figure 5.1. Results from Microcosm Studies with Subsurface Material from NAS Fallon, Showing Reduced Products from Two Repetitive Additions of Lactate (Days 0 and 80) (Bottle II-D1).

Unaccounted-for reducing equivalents from the administered lactate (i.e., the gap between equivalents fed and equivalents of products formed) was a consistent 2,500 μeq among all microcosms, regardless of the administered donor (i.e., whether lactate-fed, ethanol-fed, etc.), and did not increase with the second administration of lactate, until overpressure and repeated septum puncturing caused significant gas leakage beyond day 150. Thus, it can be reasonably inferred that unaccounted-for reducing equivalents represent the potential for reduction of sulfate (and/or possibly other electron acceptors, although groundwater and subsurface soil at NAS Fallon were known to contain very high levels of sulfate) in this system.

Note in Figure 5.1 that the H_2 level did not appear to rise until after the deficit had plateaued, i.e., until after SO_4^{2-} had been depleted. H_2 then accumulated at higher levels, and methane began to appear. It thus would seem as though the sulfate-reducers had suppressed the hydrogenotrophic methanogens; this is a commonly observed phenomenon and is a manifestation of the greater affinity for H_2 of sulfate-reducers, compared to methanogens. It would appear that acetotrophic methanogens arose concomitant with hydrogenotrophic methanogens, as evident from the depletion of acetate after about day 100 in Figure 5.1. The accumulation of propionate after the respiking event (day 80 onward) to a greater degree than after the initial feeding of lactate (i.e., days 0 through 30), is probably explained by the active sulfate reduction that occurred following the initial feeding. The sulfate-reducers kept H_2 low enough to allow propionate fermentation to acetate and H_2 .

If we compare the dechlorination profile for lactate-amended bottles in Figure 5.2 with the reduction-product/ H_2 profile of Figure 5.1, dechlorination did not seem to commence until after sulfate reduction had ceased (i.e., after day 50) but seemed to greatly increase after day 100, coincident with the onset of methanogenesis and acetate utilization. No explanation can be certain, but it is possible that sulfate reduction competitively suppressed dechlorination (i.e., via competition for H_2). The surge in dechlorination with methanogenesis could be meaningful (e.g., cometabolic dechlorination as known to occur with some species of methanogens) or coincidental, as both dechlorinators and methanogens arose following the cessation of sulfate-reducing activity.

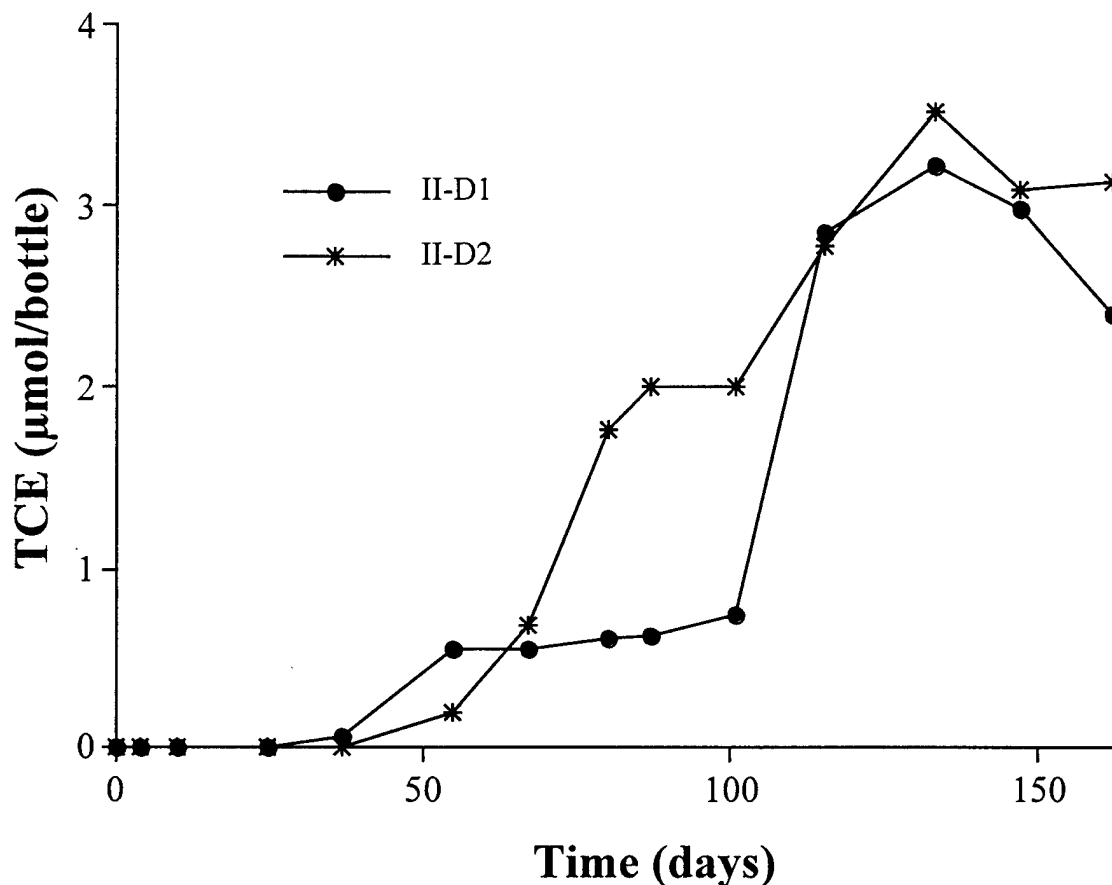


Figure 5.2. TCE Production from PCE in Two Replicate, Lactate-Fed Microcosms from NAS Fallon Study. (Bottles II-D1 and II-D2).

Such information can be used to design the injection formulation and enhancement strategy. As in the case above, the competitive demand for reducing equivalents from such activities as methanogenesis and sulfate reduction needs to be satisfied. The microcosm results can be used to assess donor fermentation pathways and the fraction of reducing equivalents that will be channeled to dechlorination. A more elegant approach is potentially available. The microcosm results (along with MPN assays, where available) can be used to develop site-specific inputs to comprehensive contaminant transport/fate models, allowing quantitative model estimates of dynamic response to alternative enhancement strategies. Currently, however, the pollutant fate and transport models that are available do not include biokinetic models that adequately describe the fate of donors and the competition for donor between different microbial groups and different TEAPs. In the absence of more sophisticated modeling approaches, a reasonable injection formulation for treatability studies may be derived as follows:

Define:

- V_T = total effective volume of treatability test plot (m^3)
 η = porosity (void-volume fraction; generally varies between 0.2 and 0.4.)
 V_{H_2O} = volume of water within the plot (m^3)
 $V_{H_2O} = V_T \cdot \eta$

 ρ_b = bulk mass density of solids within test plot (g/cm^3 of bulk volume; generally assumed to be $1.8 g/cm^3$.)
 M_{soil} = mass of dry soil solids within the plot (kg)
 $M_{soil} = 1,000 \cdot V_T \cdot \rho_b$

 Q_T = estimated flow rate actually moving through the test plot (including injected water) under test conditions (m^3/d). (Note: Q_T may be much less than the injection flow rate, if high injection rates are employed, with much of the injection water moving around, as well as through, the test plot.)
 HRT = estimated hydraulic residence time within the test plot — i.e., the time it takes for a conservative tracer to pass through the plot under test conditions (days),
 $HRT = V_{H_2O}/Q_T$

 F_R = retardation factor for chloroethenes in the test plot
 $F_R = 1 + \rho_b K_d / \eta$

Where K_d is the sorption distribution coefficient for chloroethenes (cm^3/g). Values of F_R can vary considerably from site to site, depending on organic-carbon content of the soil; a reasonable default value would be around 5 for chloroethenes.

- D_w = donor demand from contributions in the groundwater phase (mol of donor per m^3 of groundwater)
 $D_w = D_{w(ea)} + D_{w(rd)}$

Where $D_{w(ea)}$ is the donor demand from alternative electron acceptors within the groundwater phase. This can be estimated from groundwater analyses of electron acceptors, as described in Section 2.2.3 (mol of donor per m^3 of groundwater).

$D_{w(rd)}$ is the donor demand from the reductive dechlorination of dissolved chloroethenes, corrected for estimated methanogenic competition for H_2 (mol of donor per m^3 of groundwater). $D_{w(rd)}$ can be estimated from groundwater analyses of chloroethenes, and then inflated through use of a safety factor, SF_{CH_4} , for methanogenic competitive demand. It is expected that SF_{CH_4} may vary from 2 to 20, depending upon the donor selected and site-specific conditions of microbial ecology; thus,

$$D_{w(rd)} = R_{D/H_2} \cdot \{4[PCE] + 3[TCE] + 2[DCEs] + [VC]\} \cdot SF_{CH_4}$$

where the concentrations of the chloroethenes are expressed in mol per m³ groundwater, and R_{D/H_2} is the number of moles of donor required to yield one mole of H₂ in fermentation (e.g., in the case of butyrate $R_{D/H_2} = 0.5$).

$$\begin{aligned} D_s &= \text{donor demand from contributions within the soil phase (mol of donor per kg soil solids)} \\ &= D_{s(ea)} + D_{s(rd)} \end{aligned}$$

Where $D_{s(ea)}$ is the donor demand from alternative electron acceptors within the soil phase (mol of donor per kg of soil solids). This can be estimated from soil analysis using measured particulate nitrates, sulfates, and Fe(III) per kg dry soil. Bioavailability, however, may not be total. The microcosm results may be usefully employed here to estimate the concentrations of bioavailable alternative electron acceptors. Note, however, that one should correct for the fact that microcosms employ a matrix that is 50% solids and 50% groundwater (wt/wt), whereas the *in situ* aquifer is perhaps 85% solids and 15% water (wt/wt).

$D_{s(rd)}$ = donor demand from reductive dechlorination of chloroethenes sorbed to the aquifer solids within the test plot (mol of donor per kg of soil solids). The fraction of soluble chloroethenes (of the total within the test plot) can be estimated as the reciprocal of the retardation factor, F_R . Thus, by extension of what was earlier estimated for dechlorination of soluble species,

$$D_{s(rd)} = \frac{D_{w(rd)} V_{H20} (F_R - 1)}{M_{soil}}$$

A two-phased strategy is recommended for dosing the test plot:

Phase I

Phase I would utilize a higher dosing rate than Phase II, and would last for a period (τ_1) or ideally one hydraulic retention time (HRT). During this period, sufficient donor should be injected to meet the estimated demand from all soil-phase sources within the test plot (chloroethenes and alternative electron acceptors), plus the demand from all groundwater entering the plot during Phase I. The objective is to eliminate competing electron acceptors as quickly as possible, thereby establishing fermentation/methanogenic conditions as quickly as possible. Furthermore, it is recommended that the donor administered during Phase I include some readily degradable substrate (e.g., yeast extract or ethanol or lactate), as well as less-readily degradable (low-H₂-ceiling) substrate intended for later use in Phase II (to promote growth of organisms that use such substrates).

The dose may be expressed as a concentration to be achieved in the aquifer at the entrance to the test plot:

Phase I

$$\text{Donor dose conc (mol/m}^3\text{)} = D_w + \frac{D_s \cdot M_{\text{soil}}}{Q_T \cdot \tau_1}$$

In instances where a high demand for donor exists from high levels of particulate forms of alternative electron acceptors, the above-described strategy of meeting the entire particulate demand for donor within a single HRT could potentially result in inhibitory, high concentrations of donor. In such cases, Phase I must necessarily be extended, such that $\tau_1 > \text{HRT}$ (i.e., beyond a single retention time), reducing the dosage rate and concentration. In any event, Phase I represents a period in which demand for donor from particulate sources of electron acceptor exists and is dealt with.

Phase II

Phase II is the treatment period (τ_2) extending beyond Phase I, to the end of the treatment test. It is assumed that the particulate-based demand from alternative electron acceptors has been met in Phase I; however, it cannot be assumed that dechlorination activity will have arisen sufficiently in Phase I such that sorbed chloroethenes have been dechlorinated (though that *may* have occurred). Thus, a conservative dosing strategy is recommended for Phase II that targets sorbed chloroethenes (at their estimated, original levels) plus the demand for donor arising from influent groundwater (chloroethenes and alternative electron acceptors). Furthermore, the dose rate is selected to theoretically meet the demand from sorbed chloroethenes within the desired period, τ_2 .

Phase II

$$\text{Donor dose conc (mol/m}^3\text{)} = D_w + \frac{D_{s(\text{rd})} \cdot M_{\text{soil}}}{Q_T \cdot \tau_2}$$

Donor selected for Phase II should be a slowly utilized (low- H_2 -ceiling) substrate(s), to minimize methanogenic competition (or possibly a substrate whose fermentation forms such desirable substrates). Choice should be guided by the microcosm results.

6.0 FIELD TESTING

Two prerequisites must be accomplished before field testing can begin. First, a testing location must have been selected, characterized, and successfully met all technical and administrative screening criteria (see Section 4.0). Second, a microcosm study must have been conducted and the results must show successful dechlorination of targeted chloroethenes (see Section 5.0). Microcosm study results should also provide insight into electron donor selection and dosing. Having met these prerequisites, the in situ treatability test becomes a matter of designing a system for reliably distributing electron donor through the test plot and monitoring changes within the test plot. Although this task sounds deceptively easy, it is an engineering challenge that will require careful planning, design, and implementation to avoid costly mistakes.

6.1 System Design

The design and installation of the RABITT treatability test must accomplish the following objectives:

1. It must reliably distribute and direct the flow of electron donor/nutrient formulation through the test plot without displacing contaminated groundwater within the testing zone with “clean” water or solutions.
2. It must dictate a hydraulic retention time (HRT) short enough to permit electron acceptor depletion and dechlorinating activity to begin, but long enough to observe spatial changes in contaminant and electron-donor concentrations.
3. It must maintain hydraulic control while minimizing pumping requirements and the extraction of contaminated groundwater.
4. It must permit accurate and reliable sampling of amended groundwater from the treatment plot.
5. It must prevent fouling by both biological and chemical agents, including inorganic precipitates and trapped gases (e.g., methane bubbles).

6.1.1 Distribution and Direction of the Electron Donor Feed Solution. The primary challenge in the design of a RABITT treatability test system is to reliably distribute the electron donor feed solution throughout the testing zone. The proposed field treatability testing system, illustrated in Figure 6.1, distributes feed solution by forcibly injecting amended groundwater at the head of the testing zone while extracting groundwater near the end of the zone. This technique creates a hydraulic gradient designed to direct the flow of amended groundwater through the test plot. The use of three ½-inch inner diameter (ID) injection wells each equipped a 36-inch well screen should more evenly distribute the amended

groundwater across the influent face of the testing zone. The face of the testing zone will be approximately 36-inches square, so the injection wells will be spaced on 12-inch intervals.

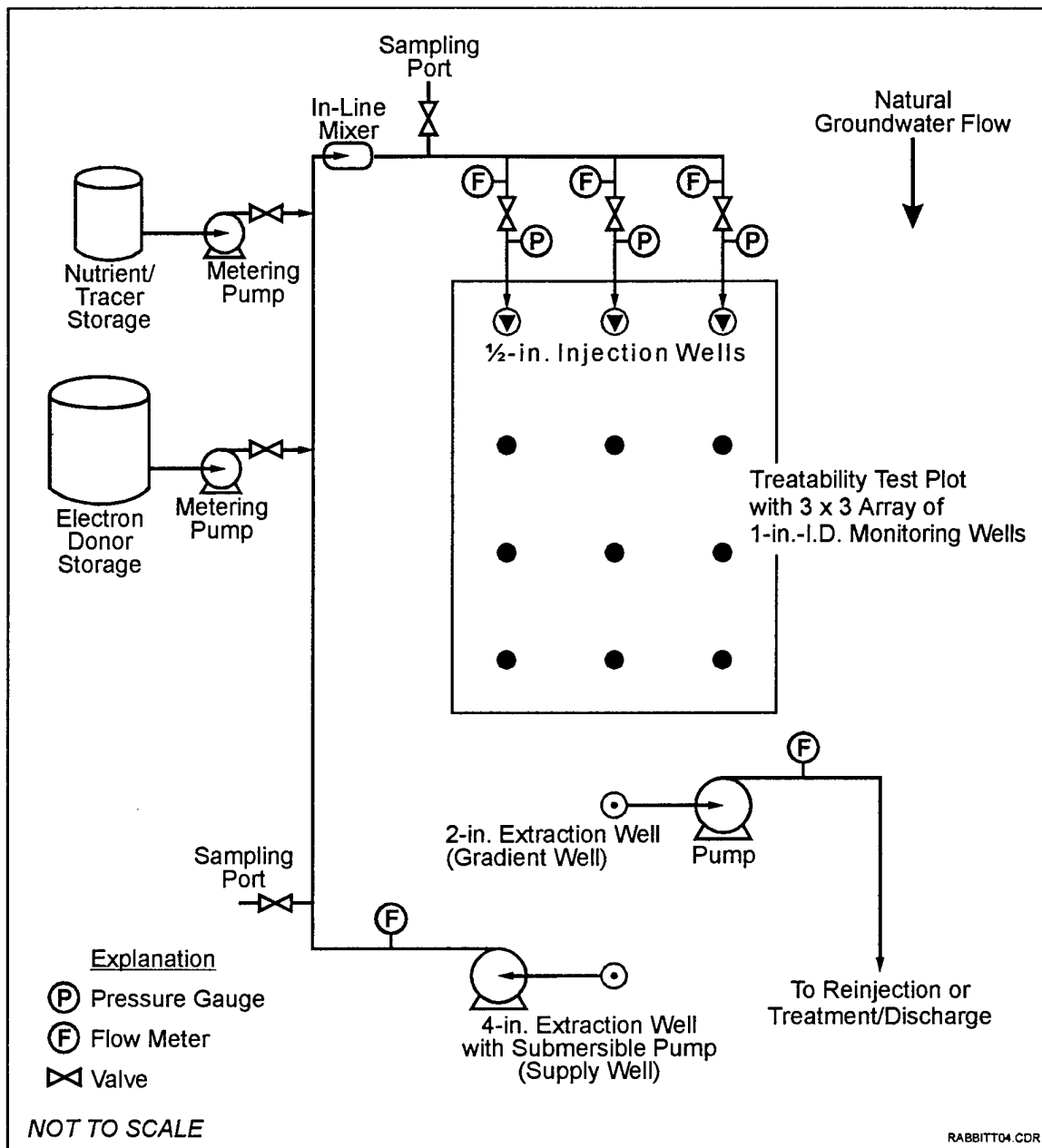


Figure 6.1. Test Plot Layout.

To avoid displacing contaminated groundwater with an uncontaminated solution, contaminated groundwater is extracted from the site, amended with electron donor and nutrients, mixed, and then injected at the influent end of the test plot. This extraction well, from hereon called the supply well, will

have a 4-inch ID and a 36-inch well screen. The test plot's influent contaminant concentration can be controlled to some degree by installing the supply well in a location with desired contaminant concentrations. Because fluctuations in contaminant concentrations will occur, periodic sampling of the groundwater extracted for reinjection will be necessary. Tracer detected in the supply well indicates that the well is in communication with the testing zone and must be abandoned. For this reason, care should be taken when selecting the location of the supply well.

6.1.2 Hydraulic Retention Time in the Testing Zone. The HRT within the test plot can be controlled by regulating the rate of groundwater injection and extraction at the influent and effluent ends of the test plot, respectively. An extraction well, from hereon called the gradient well, will be installed outside the effluent end of the testing zone. This well will have a 2-inch ID and a 36-inch well screen installed at the same depth as the injection well screens. Groundwater injection and extraction rates will be optimized with tracer testing (see Section 6.4.2) to achieve a 30-day HRT.

To accommodate the 6-month testing period, the optimum HRT between the injection wells and the last row of monitoring wells is 30 days. An HRT significantly less than 30 days may not provide a sufficient opportunity to observe changes in contaminant concentrations as a slug of groundwater passes through the testing zone. Conversely, significantly longer HRTs may not achieve steady-state conditions soon enough within the 6-month test period to allow the collection of meaningful kinetic data. In general, tests requiring 30 days for the transport of amendment throughout the treatment plot will then require an additional 60 days before steady-state conditions are likely to occur. Even then, there is no guarantee that steady-state conditions will prevail. Obviously, tests with long HRTs severely limit the length of time available to observe dechlorination reactions.

In many cases the hydraulic gradient at a site will not vary significantly within the boundaries of the relatively small treatment zone, making the groundwater direction and velocity difficult to determine accurately within the zone. In such cases, data collected throughout the site must be used to examine regional groundwater gradients, and the groundwater velocity and direction through the plot must be estimated. The plot's length and alignment may be specified from estimates of the groundwater velocity and direction. Because the HRT can be shortened only by pumping (assuming the testing zone is aligned with the extraction well directly downgradient of the injection wells), a plot length should be selected that will result in an HRT of 35 to 40 days with natural groundwater flow. For example, if the natural groundwater velocity is 0.5 foot per day (fpd), to obtain a natural HRT of 35 days the testing zone would need to be approximately 17.5 feet long. Plot lengths longer than 40 ft should be avoided

due to difficulties maintaining hydraulic control and integrity of the testing zone. In such cases, testing results may be improved by selecting a shorter HRT, on the order of 20 to 25 days. Conversely, plot lengths less than 15 feet are not recommended due to difficulties maintaining hydraulically independent boreholes during drilling and installation procedures.

As a rule of thumb the plot length can be selected based on the following criteria. Testing locations with groundwater velocities <0.5 fpd should be 15 feet long, i.e., 15 feet from the injection wells to the last row of monitoring wells. The gradient well will be placed outside the last row of monitoring points and can be used as a last monitoring location if the actual HRT within the designed testing zone is found to be insufficient. The length of testing zones with groundwater velocities >0.5 fpd can be estimated by multiplying the groundwater velocity by 35 days. For instance, a testing location with a groundwater velocity of 1 fpd would need to have a distance of 35 feet between the injection wells and the last row of monitoring wells.

6.1.3 System Alignment. Aligning system wells parallel to the natural groundwater flow will minimize pumping requirements. If groundwater flows naturally from the injection wells through the series of monitoring wells/points and to the extraction well, the need to create an artificial hydraulic gradient by extracting groundwater will be significantly reduced. Again, difficulties determining the hydraulic gradient within the relatively small test area may require the use of larger scale site data to estimate the direction of groundwater flow within the testing zone. The chances of achieving an excellent and consistent alignment with the groundwater flow direction are slim. Thus, it may be necessary to impose an artificial hydraulic gradient across the testing zone by extracting groundwater. A well-aligned system reduces, if not eliminates, the amount of pumping required from the gradient well. Because extracted groundwater probably will require aboveground treatment before it can be discharged, decreased pumping may result in significant cost reductions.

6.1.4 Monitoring Equipment. Standard monitoring wells will be used to sample the testing zone. One 1-inch ID well will be installed at each monitoring location unless the selected sampling method requires 2-inch diameter wells, in which case, 2-inch diameter wells will be substituted for the 1-inch wells. Each monitoring well will be equipped with one 18-inch well screen. The monitoring well screens should be vertically centered between the top and bottom of the injection well screens so that samples collected from the monitoring wells come from a region completely amended with electron donor. For example, a test plot with an injection well screened over the interval from 11 to 14 feet below ground surface (bgs) should be followed by monitoring wells screened from 11.75 to 13.25 feet

bgs. The accurate placement of this equipment will be imperative to guarantee that samples are taken from within the flow of amended groundwater. The use of a conservative tracer during testing will help ensure that samples are indeed being taken from within an amended section of the testing zone. Sampling the testing zone will be performed in accordance with standard sampling protocols for the analysis of VOCs.

6.1.5 Fouling. Serious fouling problems are not expected because anaerobic conditions will be maintained and the changes in redox potential and pH should not be dramatic enough to cause massive precipitation. Nonetheless, fouling problems can occur, and a means of detecting and correcting them early must be in place. The proposed testing system will monitor both head pressures and flowrates at each of the three injection wells. Routine surging will be performed when head pressures begin to increase and flowrates begin to drop. Surging should help displace biological growth, inorganic precipitates, and trapped gases (e.g., methane bubbles). In addition to surging, carefully dosing the treatment zone will help limit the production of methane, thus minimizing flow restrictions through the plot caused by trapped methane bubbles. Finally, the selection and concentration of buffer salts added to the injectate requires attention as well. For instance, phosphate buffers have been known to precipitate as $\text{Ca}_3(\text{PO}_4)_2$ in groundwater when present above 1 mg/L. An assessment of site water quality is recommended so potential precipitation reactions between inorganic species may be avoided.

6.2 Administrative Preparations

The development of a site-specific test plan, receiving regulatory approval of the test plan, and obtaining necessary permits and clearances will be necessary before the installation or use of the field treatability testing system begins. These administrative requirements are discussed in Section 4.2.

6.3 Field System Installation

The installation of field components will require the use of standard drilling and well installation methods in addition to the selection, purchase, and installation of aboveground system components. The entire installation process should be supervised and documented by an experienced field scientist/engineer, and local, state, and federal regulations governing installation and completion procedures must be followed.

6.3.1 Drilling. An appropriate drilling method must be selected based on site conditions. The discussion of standard drilling methods is outside the scope of this document; the following ASTM Methods provide detailed guidance in the selection and use of specific drilling methods:

- ASTM D 5784 *Standard Guide for Use of Hollow-Stem Augers for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1995)
- ASTM D 5781 *Standard Guide for Use of Dual-Wall Reverse-Circulation Drilling for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1995)
- ASTM D 5782 *Standard Guide for Use of Direct Air-Rotary Drilling for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1995)
- ASTM D 5783 *Standard Guide for Use of Direct Rotary Drilling with Water-Based Drilling Fluid for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1995)
- ASTM D 5872 *Standard Guide for Use of Casing Advancement Drilling Methods for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1996)
- ASTM D 5875 *Standard Guide for Use of Cable-Tool Drilling and Sampling Methods for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1996)
- ASTM D 5876 *Standard Guide for Use of Direct Rotary Wireline Casing Advancement Drilling Methods for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices* (1996)

6.3.2 Installation of Wells. Although most drilling companies provide experienced professional drilling crews, environmental systems typically require specialized supervision of the drilling process due to the more strict functional requirements of engineered flow and monitoring systems. Therefore, a qualified environmental professional or field engineer should be present to observe, supervise, and record the installation of wells. These personnel should be capable of inspecting well materials, overseeing drilling practices, and ensuring that system components are installed according to specifications. General guidance for the installation of wells is available in, *ASTM D 5092, Standard Practice for Design and Installation of Groundwater Monitoring Wells in Aquifers*.

A bound logbook documenting the entire installation, completion, and development processes should be meticulously kept. The logbook should describe the well design, give construction details, and serve as a record of well development. For example, the field engineer should verify and record drilling depths,

installed component lengths, and the well completion materials used to pack the annulus between the casing and the borehole wall. These measurements should be recorded in the logbook to the nearest 0.1 foot. Any difficulties or irregularities encountered during the drilling or installation process should be noted. Well components should be installed into the boreholes in such a manner that the as-built well matches the designed well as nearly as possible. Any deviation from the well design must be recorded in the logbook to accurately reflect the actual construction of the well. In many areas, local or state authorities require that a completed form be submitted describing any well installation made, including sketches and soil types encountered.

All wells within the RABITT testing zone should have sanitary well seals to prevent air from entering the subsurface. In addition, well screens must fall within a single contaminated and hydraulically conductive layer of the subsurface. Detailed characterization of a test plot's contaminant profiles and hydrogeologic conditions allows the accurate design and placement of system components.

6.3.3 Aboveground System Components. Aboveground system components will include storage containers, pumps, tubing, piping, fittings, valves, flow meters, pressure gauges, an in-line static mixer, sampling ports, and a source of electrical power. The general setup of aboveground components is illustrated in Figure 6.1. Because component specification requires some knowledge of site-specific conditions, general recommendations and important design considerations are discussed in this section.

Storage Considerations. A minimum of two storage containers are required, one for the electron donor feed solution and one for the tracer. One or two additional drums or containers are recommended for mixing fresh solutions.

The size of these containers will depend upon the estimated groundwater injection rate, the concentration of prepared stock solutions, the proposed groundwater-dosing rate, and the frequency of solution preparation. Higher groundwater injection rates, more dilute stock solutions, higher dosing rates, or less frequent solution preparation will require greater storage capacity. Therefore, the sizing of storage containers will require knowledge of site-specific details.

Storage containers should be selected that prevent exposure to air, sunlight, or excessive temperatures. To maintain a stable anaerobic environment in the subsurface, prepared solutions should be stripped of oxygen (by purging with nitrogen or some other suitable method) and stored in an airtight, collapsible, inert storage container. Highly concentrated biostatic stock solutions will not require oxygen stripping if the solution's oxygen solubility and feed rate are both low.

Pump Considerations. At least four pumps will be required to move groundwater and add nutrient and tracer stock solutions. Both groundwater extraction wells require one submersible pump. The sizing and power requirements of these pumps will depend upon:

- the depth to water in the extraction wells
- the required groundwater extraction rate
- the head loss through the system plumbing
- the backpressure imparted by the formation.

The head requirements associated with each of these components are estimated to calculate a total system head. Pump manufacturers provide performance curves for their pumps that plot the flow rate against the pressure head. These curves are used to select the appropriate size pump. Because the system head is an estimate, a safety factor of 50% should be considered when sizing the pump.

Chemical metering pumps are used to add the electron donor and tracer stock solutions to the groundwater as it is pumped to the injection wells. Pumps are selected that can handle the viscosity of the stock solutions. They are sized to provide the required flowrate against the system backpressure. As with the submersible pumps, pump performance curves are used to select the appropriate size pump.

Plumbing Considerations. The system's plumbing consists of tubing, piping, valves, fittings, and sampling ports. The materials selected must protect all injectate constituents (groundwater, tracer, and the electron donor/nutrient formulation) from exposure to atmospheric oxygen, sunlight and temperature extremes. In areas where temperatures drop below freezing, the lines need to be insulated and heat taped. In areas of extreme heat, the lines should be painted white, shaded, and if necessary buried.

Flexible tubing is used between the chemical metering pumps and the storage containers to allow movement during container filling and/or change out. Because the pumping rates are very low, 1/8-1/4-inch tubing usually is sufficient. The length of tubing is kept to a minimum by locating the storage container close to the metering pump. Opaque tubing such as Viton® is used to minimize light exposure. The tubing should have low gas permeability (particularly oxygen), which excludes Teflon™. Although the tubing is on the low-pressure side of the pump, thick-walled tubing is preferred.

Pipe is used to transfer groundwater and on the high-pressure side of chemical metering pumps. Polyvinyl chloride (PVC), polycarbonate, and stainless steel are the preferred materials. Iron pipe is not used due to its reactivity with the chlorinated solvents. The piping is sized to:

- accommodate the required flow rates
- minimize the HRT in the plumbing
- provide a linear velocity in the pipe that results in efficient mixing through the static mixer
- provide sufficient shear to minimize biological growth (≈ 1 foot/second).

The system should be plumbed using the maximum lengths of pipe and the minimum number of fittings. The fittings are made of the same material as the pipe. Plastic pipes and fittings are bonded together. Stainless steel or other metal pipes and fittings are threaded together using Teflon™ tape to ensure a tight seal.

Mixing Considerations. The solution containing the nutrients, tracer, and groundwater is mixed before it is injected into the aquifer. A static mixer is placed in the plumbing following introduction of the nutrient and tracer stock solutions. The mixer is sized to achieve mixing at the design flow rate.

The number of elements required is calculated using the Reynolds equation as follows.

$$Re = \frac{3157 \cdot Q \cdot S}{\mu \cdot D}$$

Where: Re = Reynolds number
 Q = Flow rate (gallons per minute)
 S = Specific gravity
 μ = Viscosity (centipoise)
 D = Inside pipe diameter (inches)

Flow Control and Monitoring Considerations. Valves are plumbed into the system to control the total flow rate, balance the flow between the three injection wells, and shut off the tracer and nutrient stock solution delivery lines. Needle valves are installed at the head of each injection well. The valves are selected to afford adequate control to balance the flow from the common manifold to each well at the design flow rate. Ball valves are installed in each of the stock solution delivery lines. Because the metering pumps control the flow rate, these valves require only open and closed positions. Stock solution feed lines should have check valves installed if the selected metering pump is not equipped with one to prevent backflow into the stock solution containers.

Groundwater and injectate flow rates are monitored and adjusted accordingly. In-line flowmeters are installed prior to the flow control valves at the head of each well, and in the groundwater supply line

near the control for the submersible pump. Rotameters are not used because of their tendency to stick when particulates, precipitates, or biological growth is formed on the float. Paddle wheel and vortex flowmeters are more appropriate but also may experience problems associated with these interferences. Magnetic flowmeters may be preferred because they are less intrusive, have no moving parts, and are not affected by viscosity. Unfortunately, magnetic flowmeters are much more expensive than other types of flowmeters. Flowmeters are selected so that the design flow rate is within 60% of the meter's range.

Pressures are measured in the delivery line to each well. Pressure gauges are selected with a minimum range of 1.5 times the expected operating pressure. The gauges are plumbed into the delivery line following the flow control valve.

Sampling Considerations. The performance of RABITT is assessed based on the changes in contaminant concentrations across the treatment plot as a function of time. It is necessary to sample the groundwater pumped from the supply well, the injectate, and groundwater from the nine monitoring wells. Sampling ports are plumbed into the system to sample the supply and injectate. The ports consist of a t-section that is inserted into the delivery line. The supply sampling port is located upstream of the stock solution injection lines, and the injectate sampling port is located downstream of the in-line mixer. The side arm of the t-section is fitted with a ball valve. The outlet downstream side of the valve is fitted with short (<6-inch) length of inert tubing to facilitate filling sample vials. The groundwater monitoring wells are not equipped with any specialized sampling features.

6.4 Field Testing Procedures

6.4.1 Phase I Injection Preparations. Before Phase I injection begins, the following design parameters must be calculated:

1. Calculate initial injection flow rate.
2. Estimate injectate travel time to individual monitoring locations.
3. Select tracer and determine desired concentration in injectate.
4. Determine maximum strength of tracer stock solution and feed rate.
5. Calculate the Phase I electron donor demand.
6. Identify Phase I electron donor(s) and calculate desired concentration in injectate.
7. Determine maximum strength of electron donor stock solution and feed rate.

6.4.1.1 Determine initial injection flow rate. The initial injection flow rate is an estimate based on the testing zone length, the injection well screen size, and the HRT. The testing zone length, injection well screen size, and formation porosity are used to calculate the approximate volume of aquifer that

could be impacted by the injection. For simplicity, this volume is assumed to be a cylinder with a radius equal to the length of the testing zone and a height equal to the injection screen length. The flow rate is estimated by dividing the void volume of that cylinder by the HRT (30 days) and multiplying by a safety factor of 1.5. The void volume must be filled in 30 days to ensure the last set of monitoring wells receives injected groundwater and the safety factor will accommodate any flow of injectate out of the calculated cylinder volume. An example calculation is supplied below for testing locations with groundwater velocities ≤ 0.5 fpd.

Testing systems at locations with groundwater velocities ≤ 0.5 fpd will be 15 feet long and have 3-foot-long injection well screens. The desired initial flow rate (Q_I) will fill the void space in a 15-foot radius around the injection well screen in 30 days. The effects of natural groundwater flow will be neglected in lieu of pumping adjustments made during tracer testing.

The calculation goes as follows:

Step 1: Calculate the void volume (V_v) of a cylinder with a radius (r) of 15 feet, a height (h) of 3 feet, and a porosity (η) of 0.3:

$$\begin{aligned} V_v &= \eta \cdot h \cdot \pi \cdot (r)^2 \\ V_v &= (0.3) \cdot (3 \text{ ft}) \cdot \pi \cdot (15 \text{ ft})^2 \\ V_v &= 636.17 \text{ ft}^3 \text{ or } 4,759 \text{ gal} \end{aligned}$$

Step 2: Calculate the injection flow rate required to fill the void volume in 30 days.

$$Q_I = \frac{V_v}{\text{HRT}} = \frac{4,759 \text{ gal}}{30 \text{ days}} \cong 159 \text{ gpd or } 0.11 \text{ gpm}$$

Step 3: Multiply the calculated initial injection rate (Q_I) by a safety factor of 1.5.

$$Q_{sf} = 1.5 \cdot Q_I = 0.165 \text{ gpm}$$

6.4.1.2 Estimate injectate travel time to monitoring locations. Because the injectate is assumed to move more or less radially from the injection wells, its travel time to the first monitoring point will be shorter than the travel time from the first monitoring point to the second, even though the monitoring points are evenly spaced. Therefore, the following equation is supplied to approximate the travel time (t) to a point some radial distance (r_d) from an injection well to determine if the initial injection rate is on target for a 30-day HRT:

$$t = \pi \cdot \eta \cdot h \cdot (r_d)^2 / Q_I$$

For example, a treatability test such as the scenario described in Section 6.4.1.1 which has an injection rate (Q_I) of 21.2 cfd (0.11 gpm), a porosity (η) of 0.3, and a screen length (h) of 3 ft the equation simplifies to:

$$t = (r)^2 \cdot 0.133 \text{ day/feet}$$

Assuming the 15-foot-long testing zone has three sets of monitoring wells at 5, 10, and 15 feet from the injection wells, the travel times to each set of wells would be 3.3, 13.3, and 30 days, respectively. Based on this calculation one should expect to see tracer breakthrough at the first row of monitoring points on the third day of injection. If breakthrough occurs sooner, which is likely due to the use of the safety factor, the flow rate can be decreased proportionately. If tracer does not break through by the end of Day 3, the injection flow rate should be increased by 10% each day until tracer is detected. That is, the injection flow rate will be increased to 110% of the initial injection rate on Day 4 and to 120% of the initial injection rate on Day 5, assuming tracer breakthrough was not observed on Day 4. If tracer has not reached the first row of monitoring wells by twice the expected travel time, the test plot may not be properly aligned with the groundwater flow direction, and extraction from the gradient well should begin.

Unless more than one tracer is used, it will be necessary to change the injected tracer concentration each time the flow rates are adjusted. Generally, increasing the tracer concentration by 50% of the initial concentration for the first two pumping adjustments should allow the observation the new tracer-travel time. If a third adjustment becomes necessary, drop the tracer concentration to its initial level. This sequence of concentration changes may be repeated as additional pumping adjustments are made. New travel times are demonstrated by the breakthrough of additional tracer fronts (see Section 7.1 for tracer data interpretation).

6.4.1.3 Select tracer and concentration. Numerous compounds may be suitable for use as tracers. Some of the more common tracers used in groundwater investigations include bromide, chloride, rhodamine, fluorescein, and sulfur hexafluoride (SF_6). Other less commonly used tracers include other dyes, alcohols, and fresh water. Each of these tracers has distinct advantages and disadvantages. The selection of the tracer to use at any given site is dependent upon the geochemistry of the groundwater and the desired property of the tracer.

Bromide and chloride are easy tracers to use for field investigations because they require no special handling procedures and are easy to measure in the field using either a conductivity meter or an ion-specific electrode. Conductivity is a quick and easy method for monitoring, but it may take an undesirable amount of salt to raise the conductivity to detectable levels. The ion-specific electrode may be preferred, but it is important to determine if there are any interfering ions in the groundwater prior to planning this method of detection. The background concentrations of both chloride and bromide will be the most important factors in determining if these tracers are applicable at a given site.

The selected tracer should be injected at a concentration at least 10 times background concentration or 100 times greater than the tracer's detection limit, whichever is greater. This concentration should be determined before the tracer is selected to make certain the desired concentrations are practical. For example, the amount of chloride needed in a slightly brackish aquifer may be enough to inhibit indigenous microorganisms.

6.4.1.4 Determine strength of tracer stock solution and feed rate. Once the tracer has been selected and the target injectate concentration has been set, the concentration of the tracer stock solution and the feed rate necessary to achieve the target injectate concentration can be determined. To minimize storage requirements and the potential for microbial contamination, tracer stock solutions should be prepared at the highest concentration possible that does not cause pumping or storage problems. Problems associated with precipitation can be encountered if concentrations near the tracer's solubility limit are selected. High-strength solutions of sodium chloride (≈ 250 g/L) or sodium bromide (≈ 310 g/L) will inhibit microbial growth and preclude the use of preservatives.

Once the stock solution strength has been specified, the stock solution feed rate can be determined based on the initial injection flow rate and the desired injectate concentration. The following mass balance equation can be used:

$$Q_{sf} \cdot C_{sf} = Q_{tr} \cdot C_{tr} + Q_s \cdot C_s$$

Where:

Q_{sf} = the initial injection rate with safety factor included (gpm)

C_{sf} = the tracer concentration in the injectate (mg/L)

Q_{tr} = the flow rate of concentrated tracer stock solution (gpm)

C_{tr} = the concentration of the tracer stock solution (mg/L)

Q_s = the flow rate of groundwater from the supply well (gpm)

C_s = the concentration of tracer in groundwater from the supply well (mg/L)

6.4.1.5 Estimate the Phase I electron donor demand. The Phase I electron donor demand can be estimated by two methods. The first method uses site characterization data to estimate the total quantity of electron acceptor within the testing zone. The second method uses microcosm results to determine the quantity of electron donor required to achieve dechlorinating conditions. Both methods must determine the aqueous phase concentration of chloroethenes and aqueous phase electron acceptors entering the testing zone at the injection wells. This is easily accomplished by sampling the groundwater extracted from the supply well for chloroethenes, dissolved oxygen, nitrate, and sulfate.

The major drawback to the site characterization method is the assessment of the solid-phase electron donor demand. Because the bioavailability of certain particulate electron-accepting species is difficult to assess, this method provides a very rough estimate of solid-phase demand. Nonetheless, the site characterization method, discussed in Sections 2.2.3 and 5.4, does provide a valid starting point for selecting an appropriate electron donor dosage.

The microcosm method does account for the bioavailability of particulate forms of electron acceptors and generally provides a more accurate assessment of the electron donor demand. This method provides information about the relationships between electron donor utilization, the predominant TEAP, and dechlorination. The total demand demonstrated by the microcosms can be scaled up to estimate the demand in the field. An example of how microcosm results can be interpreted to assess electron donor demand and the relationship to dechlorination is provided in Section 5.4. Because the microcosms are run in batch, they do not account for the additional demand exerted by the influent groundwater. For this reason, the supply well is sampled and analyzed for electron accepting species as described above.

6.4.1.6 Identify Phase I electron donor(s) and calculate desired concentration in injectate. Several factors will influence the selection of the electron donor. Donors exhibiting the most rapid and most complete dechlorination during microcosm studies will be preferentially selected, but other factors that must be considered include the rate of electron acceptor depletion, cost, and the percentage of reducing equivalents used for dechlorination versus other electron donor sinks.

The concentration of electron donor in the injectate will be a function of the electron donor demand. During Phase I injection, the electron donor dose must be sufficient to meet both the aqueous- and solid-phase demands in approximately one HRT. Once the donor demand is calculated (see Section 6.4.1.5), determining the dose is a simple matter of dividing the demand by one HRT or 30 days.

6.4.1.7 Determine maximum strength of electron donor stock solution and feed rate. The stock solution of the electron donor/nutrient formulation should be prepared at a strength where the water activity and/or pH are low enough to prevent microbial growth. Honey is a good example of a common organic substance with low water activity; it does not require refrigeration and yet does not spoil. Depending on the exact formulation, it may be necessary to separate components of the formulation to prevent any precipitation from occurring with the less-soluble ingredients. Preparing separate solutions has the advantage that, if one of the solutions becomes contaminated, it is not necessary to replace the entire formulation, saving chemical and labor costs. The disadvantage is that separate solutions require more pumps and involved injection monitoring.

Typically, the electron donors can be prepared separately at a high enough concentration to prevent microbial growth. Ethanol can be stored as a pure solvent to ensure that no growth will occur. Lactic acid and butyric acid can be prepared as very strong stock solutions. Benzoic acid on the other hand has a lower solubility (approximately 2.9 g/L), so if this electron donor is selected, pH adjustment may be the best method for preserving this solution. Coincidentally, benzoic acid has antimicrobial properties and is used as a food preservative at a concentration of 0.1%; consequently, lowering the pH of this solution will be a particularly effective method of preservation. The selection of yeast extract as an electron donor will require special considerations because it cannot be prepared in a solution with a sufficiently low water activity to inhibit microbial growth and lowering the pH may denature the solution components. In such cases, the use of a preservative, perhaps benzoic acid, could be used. The preservative concentration used must be carefully balanced so it inhibits growth in the stock solution, but is dilute enough in the injectate to have no effect on subsurface microorganisms.

Table 6.1. Solubilities of Typical Electron Donor/Nutrient Formulation Components

Formulation Component	Solubility
Electron Donors	
Ethanol	Miscible ¹
Lactic acid (available in syrup form)	Very soluble ¹
Butyric acid	Miscible ¹
Benzoic acid	2.9 g/L ¹
Yeast extract	Not available
Vitamins	
Vitamin B ₁₂ (cyanocobalamin)	12.5 g/L ²
Buffer Salts	
NaHCO ₃	100 g/L ²
Na ₂ HPO ₄	125 g/L ²
NaH ₂ PO ₄ •H ₂ O	71 g/L ¹

1. Dean, J.A. 1992. *Lange's Handbook of Chemistry*.

2. Stecher et al. 1968. *The Merck Index*, 8th Edition.

The mass balance calculation for determining the electron donor stock solution feed rate is identical to the calculation used to determine the tracer stock solution feed rate in Section 6.4.1.3. The electron donor stock solution concentration and flow rate are substituted for the tracer stock solution concentration and flow rate.

6.4.1.8 Prepare stock solutions. Once the appropriate concentration and corresponding volumetric flow rate of stock solutions have been determined, the solutions should be prepared. The preparation of large quantities of the formulation will minimize the labor costs associated with frequent preparation of new solution batches. Typically, batches of up to 50 gallons can be prepared in lined 55-gallon drums. The stock solution(s) should be prepared by adding the ingredients to their respective container. The contents of the containers are thoroughly mixed. Care should be taken to prevent aerating the solutions, thereby increasing their oxygen content. If the stock solution maintains high levels of dissolved oxygen ($> 5 \text{ mg/L}$), it should be purged with nitrogen gas and stored under a nitrogen headspace or in a collapsible, airtight container. The turbulent movement of nitrogen bubbles during purging can be used to mix the solution.

6.4.2 Phase I Injection: Tracer Testing and Electron Acceptor Depletion. The objectives of Phase I injection include the optimization of pumping rates to achieve a 30-day HRT, the depletion of electron acceptors, and the acclimatization of the subsurface microbial ecology. These objectives will be achieved concurrently by simultaneously injecting the conservative tracer and electron donor solutions. Phase I will be complete when sulfate-reducing or methanogenic conditions are established in the last row of monitoring wells and an acceptable HRT (≈ 30 days) has been demonstrated by tracer testing. The production of hydrogen sulfide can be used as an indicator of sulfate reduction, and the production of methane can be used as an indicator of methanogenesis. Once each of these criteria have been met, Phase II injection may begin. Ideally, this work would be completed in approximately 45 days.

The Phase I injection strategy follows the five steps outlined below:

- Step 1: Begin extracting groundwater from the supply well at the desired injection rate and inject the groundwater through the injection wells. Do not begin metering in stock solutions at this time. If necessary, adjust pumping rates to achieve the desired flow rate and monitor system pressures and flow rates for unexpected changes. Allow the system to run for a full day without adjustment to establish that it is operating at steady state. Check the system for failures, leaks, or other technical problems. If necessary, bleed air out of system pipes and tubing.

- Step 2: Initiate the system monitoring protocol described in Section 6.4.4 by taking the first round of samples.
- Step 3: Begin metering in the stock solutions of tracer and electron donor at the predetermined feed rates.
- Step 4: Monitor the flow of tracer through the testing zone and compare the actual tracer travel time to the expected travel time. If necessary, adjust the pumping rate so the final HRT will be approximately 30 days; this may require the extraction of groundwater from the gradient well (see Section 6.4.1.2.). In addition to tracer monitoring, geochemical monitoring should be ongoing. Changes in electron acceptor concentrations and the production of hydrogen sulfide and methane gases should be expected.
- Step 5: Operate system continuously until sulfate-reducing or methanogenic conditions are established in the last row of monitoring wells and the HRT is approximately 30 days. Then proceed to Phase II injection.

6.4.3 Phase II Injection: Steady-State System Operation By the end of Phase I injection, the HRT has been established at approximately 30 days and the vast majority of solid-phase electron acceptors have been exhausted from the testing zone. The objective of Phase II injection is to establish steady-state conditions in situ. Now that the solid-phase electron acceptors have been depleted, the electron donor dose should be decreased to prevent overdosing the testing zone. The new dose should satisfy the aqueous-phase chloroethene and electron acceptor demand and maintain a steady culture of methanogens. The Phase I dosing strategy should be revisited and based on previous calculations, microcosm results, and field observations, and an appropriate dose reduction should be made.

The Phase II injection strategy follows the three steps outlined below:

- Step 1: Reduce the electron donor dosage to account for the depletion of solid-phase electron acceptors.
- Step 2: Continue monitoring according to the system monitoring protocol described in Section 6.4.4.
- Step 3: Operate system continuously for the remainder of the 6-month testing period.

6.4.4 System Monitoring Protocol. During system operation, groundwater samples will be routinely taken from each of the nine monitoring wells, and from sampling ports located on the supply well effluent line and just after the static mixer (see Figure 6.1 for sampling port locations). Proper sampling procedures must be followed to ensure the collection of representative samples (see Section 4.3.4).

The suite of analysis outlined in Table 4.2 will be used on all collected groundwater samples taken during testing. In addition to the methods listed in Table 4.2, electron donor and electron donor fermentation products will be monitored during field-testing. The analytical methods used to monitor these compounds are described in Sections 5.3.2.2 and 5.3.2.3.

The methods listed in Table 4.2 are standard methods that are accepted by EPA and are performed by most contract analytical laboratories. Alternative methods can be used, provided that their precision and accuracy has been demonstrated and that they are approved by the appropriate regulatory agency. It is important to use the same analytical methods, and if possible the same analytical laboratory, throughout the treatability test to maintain consistency in the procedures followed and the data that result from the analyses.

The initial round of samples will be collected from all sampling locations at system startup, time zero. During Phase I injection, samples will be collected from the supply line, the injectate line, and each monitoring well at least weekly. In addition, each monitoring well will be sampled for tracer 2 days before expected tracer breakthrough, and each consecutive day thereafter until tracer breakthrough is observed. Phase II sampling will continue with weekly sampling events unless it is determined that less frequent sampling would be sufficient.

6.4.5 System Maintenance. Routine system maintenance entails visual inspection of all system components and monitoring of system pressures and flow rates. Any system component showing early signs of wear should be serviced or replaced before catastrophic failure. Thorough inspections and effective monitoring help to minimize downtimes. It is especially important when conducting the RABITT treatability test that downtime be minimized to avoid perturbations to the microbial processes driving the reductive dechlorination reactions.

Routine visual inspections involve examining the condition of all storage containers, tubing, pumps, piping and connections, valves, flowmeters and pressure gauges. Storage containers are inspected for leaks and structural integrity. If found to be in a deteriorated condition, the containers are replaced. Pumps are checked and if leaking, noisy, or drawing increased amperage, they are serviced or replaced. Tubing that shows signs of cracking, breaking, or oxidation is replaced. Leaking pipes and/or fittings are tightened and if necessary replaced.

Trends between system flow and pressure are indicative of the status of various system components. Flow decreases accompanied by decreases in pressures indicate slippage of the control valves at the well head, deterioration of pumps, plumbing leakage on the upstream side of the pump, and/or fouling of the supply well screen. Flow decreases accompanied by increased pressures indicates an obstruction in the system plumbing after the pressure gauge, clogging of the injection well screens, and/or clogging of the formation. Increased flow with no change or a decrease in pressure can indicate system leakage downstream from the pressure gauge, or a decrease in system pressure due to lower water table elevations. Increased flow coupled with increased pressures can indicate the need for pump adjustment or a problem with either or both the flow meter and pressure gauge.

7.0 DATA ANALYSIS AND INTERPRETATION

The data collected during the treatability test should be tabulated and graphed to observe trends in relevant groundwater parameters. Data collected at each monitoring location should be compiled to provide an overview of the changes that occurred throughout the test plot. In addition, a statistical analysis should be performed to determine if observed changes in measured concentrations are statistically significant. These changes must be compared to the variation observed in the water extracted from the supply well. A t-test with a 5% significance level ($\alpha = 0.05$) can be used to compare the mean value of measured concentrations from separate sampling events. Furthermore, graphed data should be plotted with error bars that represent a 95% confidence interval. The following sections discuss qualitative interpretations of specific data types.

7.1 Tracer Data.

The tracer data is plotted to determine both the travel time between the injection well and each monitoring well location. The tracer concentration is plotted against time and should produce a standard breakthrough curve. The travel time can be defined as the time at which the tracer first appears at the monitoring point, as the time of the point of inflection on the tracer curve, or as the time that the steady state tracer concentration is achieved. For the purposes of determining kinetic constants with RABITT, the time to inflection should be used as the travel time. The communication efficiency is calculated as the percent of the injected tracer recovered at each monitoring well as follows:

$$C_{\text{eff}} = \frac{C_{\text{inj}} - C_{\text{MW}}}{C_{\text{inj}}} \times 100$$

Where: C_{eff} = communication efficiency

C_{inf} = tracer concentration in the injectate

C_{MW} = steady state tracer concentration in the monitoring well.

Consistent levels of tracer at a monitoring location indicate a constant level of hydraulic communication between the injection wells and that point. The greater the level of tracer recovery, the better the hydraulic containment within the test plot. Plots with highly variable tracer recovery will require more involved data interpretation. Samples containing <50% of the initial tracer concentration should be used

with caution, because the majority of the sample was contributed from background flow, not the injectate.

A thorough discussion of tracer testing is presented in Levenspiel (1972).

7.2 Chloroethene Data.

Because the reductive dechlorination of chloroethenes is the primary goal of this treatability test, data describing changes in their concentrations will be of primary interest. The reductive dechlorination pathway follows a known sequence of transformations (see Figure 2.1); consequently, they are easy to recognize. The initial step in the process is the removal of a single chlorine atom from the PCE molecule to form TCE which is then transformed into DCEs which in turn is transformed into VC. In some instances, the rate of TCE dechlorination may rival the rate of PCE dechlorination, in which case only small amounts of TCE may be detected before the concentration of DCEs begin to rise. Because the transformation rates of DCEs are considerably slower than for PCE and TCE, DCEs will begin to accumulate before they are further transformed to VC and finally ethene. Qualitatively, the production, accumulation, and subsequent depletion of DCEs and VC within a test plot demonstrate a strong potential for RABITT success at a site.

Although qualitative contaminant data are easily examined, obtaining reliable kinetic rate data from the treatability study may be a more difficult task for several reasons. First, the microbial consortium responsible for dechlorination may not be uniform across the plot; therefore, the time the contaminant is exposed to a dechlorinating population cannot be truly defined. Achieving a uniform population throughout the plot within the 6-month period may not be possible. Second, the lack of strict hydraulic containment may cause significant changes in contaminant concentrations, particularly following rainfall events at shallow sites. Finally, influent contaminant concentrations may fluctuate considerably. Although these will be measured prior to electron donor addition, one cannot be certain the same slug of groundwater is being sampled repetitively as it passes through the testing zone unless travel times between wells are very well defined.

Despite these limitations, dechlorination rate estimates are calculated using the difference in concentrations between samples of the injectate and each water from each monitoring well, and the travel times to those monitoring wells. Data from samples containing < 50% of the tracer are used with caution because the majority of the sample originated from outside the injectate and the actual initial

concentrations are not known. Samples displaying higher levels of tracer recovery will provide more reliable rate data. Methods for estimating rate constants for sequential reductive dechlorination reactions are discussed in Corapcioglu and Hossain (1991).

7.3 Ethene and Ethane

The reduction of VC to ethene is the last step in the RABITT process. Sites demonstrating significant ethene production and the simultaneous reduction of VC concentrations are particularly good candidates for the implementation of RABITT.

Although not an intended goal of RABITT, the reduction of ethene to ethane is a possibility at sites exhibiting extremely reducing conditions. Although this transformation will consume reducing equivalents, a laboratory study conducted in the Netherlands did not observe the reduction of ethene to ethane until the VC concentrations had been nearly exhausted (de Bruin et al., 1992). Because the depletion of VC signals the end of the RABITT process, the reduction of ethene to ethane should not be a concern.

7.4 Methane

The production of methane is a clear indicator of methanogenic conditions in the subsurface. Although methane production demonstrates a depletion of available electron acceptors, it also signals the beginning of competition for reducing equivalents between dechlorinating and methanogenic organisms. Constantly increasing levels of methane production indicate that a large portion of supplied reducing equivalents is being utilized by methanogens. This situation likely will continue until methanogens out compete dechlorinating species and begin using all available reducing equivalents. The resulting methane production will be steady and very high.

7.5 Electron Acceptor Data

Electron acceptor concentrations should decrease in sequence as electron donor is added to the test plot. A rapid decrease in O_2 concentrations (to < 0.5 mg/L) should be followed by a decrease in NO_3^- (to < 1 mg/L), an increase in Fe(II), and finally a decrease in SO_4 . Each of these parameters should stabilize at a relatively low concentration with the exception of Fe(II), which will stabilize at a value dependent on

the concentration of bioavailable iron in the aquifer. Areas with little to no bioavailable iron will not display an observable increase in Fe(II) concentrations.

Test plots that maintain elevated concentrations of any electron acceptor and do not demonstrate active dechlorination must be reexamined to determine if increasing the electron donor dose will alleviate the problem.

7.6 Final Technology Assessment

After collecting and analyzing RABITT test data, the site-specific feasibility of using RABITT can be assessed. Because RABITT applicability will be defined by technical and administrative project goals, costs, and regulatory constraints, the final decision to implement or exclude the technology should result from examining test results in light of these project-specific criteria.

A clearly defined list of project goals should be compiled and compared with test results. These goals should include the minimally acceptable level and rate of contaminant destruction. The rate and extent of dechlorination observed during the treatability test must be sufficient to achieve these goals within the time frame of the project. In instances where DCE or VC accumulate and persist within the 6-month test period, the data need to be scrutinized carefully for evidence (e.g., the production of ethene) that longer treatment times would effect complete dechlorination. When such evidence is lacking, consideration needs to be given to coupling RABITT to other technologies capable of completing the destruction of residual daughter products. If the levels or rates of dechlorination observed in the test plot do not meet the goals set forth by the project, and no strong evidence exists to suggest that treatment levels or rates will improve with time or with the coupling to another technology, RABITT should be excluded from further consideration at that specific site.

The issue of cost also must be evaluated before deciding to proceed to pilot- or full-scale implementation. The cost of implementing RABITT will vary widely among sites, so a cost benefit analysis needs to be performed for each site under consideration. For instance, sites outfitted with existing pump-and-treat systems would require significantly less capital investment because RABITT can be coupled to existing wells that are already installed throughout the plume. At such sites, the benefit of incomplete dechlorination may outweigh the costs. If hydraulic control of the plume is necessary, the cost of treating extracted contaminated groundwater could be considerable. However, if

regulatory approval can be obtained to recycle groundwater between extraction and injection wells, these costs can be mitigated.

The final decision to proceed with pilot- or full-scale implementation of RABITT must consider technical and administrative project goals as well as regulatory constraints and the results of a site-specific cost benefit analysis. Evaluating the data collected from the treatability test with these criteria in mind will allow an informed assessment of the potential for using RABITT at a specific site.

8.0 SCALE-UP CONSIDERATIONS

The test results obtained from the treatability study described in this protocol are used to screen out sites from consideration for application of RABITT. The decisions are based on the contaminant distribution, the geochemical and hydrogeologic constraints, and the ability of RABITT to achieve a desired target level of contaminant reduction during treatability testing. If the results from the four-phase test described in this protocol indicate that the RABITT process is appropriate for a given site, the next step to consider is pilot-scale testing or full-scale application. Proceeding directly to full-scale implementation should be considered only for small sites, sites that have been thoroughly characterized with respect to their hydrogeologic and geochemical properties, or sites with an ongoing remediation effort to which RABITT could be directly coupled. In cases where the plumes are large and the site hydrogeology and geochemistry are not fully understood, it is necessary to run pilot tests to effectively design a full-scale system.

Although the optimum application of RABITT would result in complete dechlorination of the chloroethenes, the data from the treatability test may indicate that the process is capable of dehalogenating chloroethenes only to an intermediate level, resulting in the accumulation of the DCEs or VC. Because these are not desirable end products of chloroethene bioremediation, it may be necessary to couple an additional technology to RABITT to remove any accumulated products to achieve treatment goals.

For example, if DCE is the primary end product of the RABITT process, natural attenuation may be a plausible technology to complete the remediation process. The first two phases of the treatability test should provide data to determine the potential success of natural attenuation. If natural attenuation is not appropriate, a more aggressive removal technology such as air sparging combined with soil vapor extraction (SVE), or in situ chemical oxidation may be employed. If VC is the primary end product, natural attenuation may have potential and should be screened using the data from the first two phases of the treatability study described in this protocol. As with DCE, if natural attenuation is not appropriate, reaeration of the aquifer may be appropriate using technologies such as air sparging or use of oxygen release compounds (ORCs) to complete the remediation process.

If a coupled technology is required, a treatability test should be conducted to verify the potential for treatment before going to pilot-scale testing or full-scale application. The test can be run by installing the technology on the downgradient end of the test system used in the fourth phase described in this

protocol. The objective of the treatability test is to gain the data necessary to evaluate the effectiveness of coupling the technologies before proceeding to pilot-scale testing or full-scale application.

Pilot-scale testing typically is conducted to collect data required for scale-up of a process to full-scale application. With regards to RABITT, pilot-scale testing focuses primarily on defining the hydrogeologic properties within the contaminated volume of an aquifer. This requires a more thorough site investigation to better define the vertical extent of the contamination, the stratigraphy, and the hydraulic properties such as groundwater flow direction, velocity and hydraulic conductivity. The data necessary for running aquifer response models may be necessary for larger plumes that require significant manipulation of groundwater flow. The models are used to properly design a network of injection and extractions wells that will effect delivery of electron donor formulation throughout the contaminated volume.

Many sites may already be undergoing remediation using an alternative technology such as pump and treat. At these sites, it may be possible to couple RABITT to a portion of the existing treatment system for pilot-scale testing to evaluate the potential for enhanced remediation. If significant enhancement occurs, it may be easy to expand RABITT to the remainder of the existing treatment system.

9.0 REFERENCES

- American Public Health Association, American Water Works Association, and Water Environment Federation. Standard Methods for the Examination of Water and Wastewater, 18th edition, 1992
- American Society for Testing and Materials (ASTM). 1997. Method D 6001, Direct-Push Water Sampling for Geoenvironmental Investigations.
- American Society for Testing and Materials (ASTM). 1997. Method D 4044, Test Method (Field Procedure) for Instantaneous Change in Head (Slug Tests) for Determining Hydraulic Properties of Aquifers.
- American Society for Testing and Materials (ASTM). 1996. Method D 5730, Standard Guide for Site Characterization for Environmental Purposes With Emphasis on Soil, Rock, the Vadose Zone and Groundwater.
- American Society for Testing and Materials (ASTM). 1996. Method D 5872, Standard Guide for Use of Casing Advancement Drilling Methods for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1996. Method D 5875, Standard Guide for Use of Cable-Tool Drilling and Sampling Methods for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1996. Method D 5876, Standard Guide for Use of Direct Rotary Wireline Casing Advancement Drilling Methods for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1995. Method D 5784, Standard Guide for Use of Hollow-Stem Augers for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1995. Method D 5781, Standard Guide for Use of Dual-Wall Reverse-Circulation Drilling for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1995. Method D 5782, Standard Guide for Use of Direct Air-Rotary Drilling for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1995. Method D 5783, Standard Guide for Use of Direct Rotary Drilling with Water-Based Drilling Fluid for Geoenvironmental Exploration and Installation of Subsurface Water-Quality Monitoring Devices.
- American Society for Testing and Materials (ASTM). 1990. Method D 5092, Standard Practice for Design and Installation of Groundwater Monitoring Wells in Aquifers.
- American Society for Testing and Materials (ASTM). 1986. Method D 4448, Standard Guide for Sampling Groundwater Monitoring Wells.

- Ashworth, R.A., G.B. Howe, M.E. Mullins, and T.N. Rogers. 1988. "Air-Water Partitioning Coefficients of Organics in Dilute Aqueous Solutions." *Journal of Hazardous Materials* 18: 25-36.
- Bagley, D.M., and J.M. Gossett. 1990. "Tetrachloroethene Transformation to Trichloroethene and cis-1,2-Dichloroethene by Sulfate-Reducing Enrichment Cultures." *Applied and Environmental Microbiology* 56(8): 2511-2516.
- Barrio-Lage, G., F.Z. Parsons, and R.S. Nassar. 1987. "Kinetics of the Depletion of Trichloroethene." *Environmental Science and Technology* 21(4): 366-370.
- Becvar, E., Vogel, C., Sewell, G., Gossett, J., Zinder, S., and Magar, V. 1997. "In Situ Dechlorination of Solvents in Saturated Soils." *Fourth International In Situ and On-Site Bioremediation Symposium* 3: 39-44. New Orleans, LA.
- Beeman, R.E. 1994. "In Situ Biodegradation of Groundwater Contaminants." U.S. Patent No. 5,277,815.
- Bradley, P.M. and F.H. Chapelle. 1996. "Anaerobic Mineralization of Vinyl Chloride in Fe(III)-Reducing Aquifer Sediments." *Envir. Sci. Technol.* 30: 2084-2086.
- Brusseau, M.L. 1993. *Complex Mixtures and Groundwater Quality*. EPA/600/S-93/004. U.S. Environmental Protection Agency.
- Chapelle, F.H. and P.B. McMahon. 1991. *J. Hydrol.* 127: 85-108.
- Chapell, F.H. 1993. *Ground-Water Microbiology & Geochemistry*. John Wiley and Sons, Inc. New York, NY.
- Chu, K.H., and W.J. Jewell. 1994. "Treatment of Tetrachloroethylene with Anaerobic Attached Film Process." *Journal of Environmental Engineering* 120(1): 58-71.
- Corapcioglu, M.Y., and M.A. Hossain. 1991. "Estimating Biotransformation Rate Constants for Sequential Reductive Dechlorination Reactions." *Journal of Environmental Engineering* 117(5): 631-639.
- Dean, J.A.(Ed.) 1992. *Lange's Handbook of Chemistry*. 14th Edition. McGraw-Hill, Inc. New York, NY.
- de Bruin, W.P., M.J.J. Kotterman, M.A. Posthumus, G. Schraa, and A.J.B. Zehnder. 1992. "Complete Biological Reductive Transformation of Tetrachloroethene to Ethane." *Applied and Environmental Microbiology* 58(6): 1996-2000.
- DiStefano, T.D., J.M. Gossett, and S.H. Zinder. 1991. "Reductive Dechlorination of High Concentrations of Tetrachloroethene to Ethene by an Anaerobic Enrichment Culture in the Absence of Methanogenesis." *Applied and Environmental Microbiology* 57(8): 2287-2292.
- DiStefano, T.D., J.M. Gossett, and S.H. Zinder. 1992. "Hydrogen as an Electron Donor for Dechlorination of Tetrachloroethene by an Anaerobic Mixed Culture." *Applied and Environmental Microbiology* 58(11): 3622-3629.

- Domenico, P.A., and F.W. Swhartz. 1990. Physical and Chemical Hydrogeology. John Wiley and Sons. New York, NY.
- Fennell, D.E. 1998. Comparison of Alternative Hydrogen Donors for Stimulation of Tetrachloroethene Dechlorination. Ph.D. Dissertation. Cornell University. Ithaca, NY.
- Fennell, D.E., J.M. Gossett, and S.H. Zinder. 1997. "Comparison of Butyric Acid, Ethanol, Lactic Acid, and Propionic Acid as Hydrogen Donors for the Reductive Dechlorination of Tetrachloroethene," *Environmental Science & Technology* 31: 918-926.
- Fennell, D.E., M.A. Stover, S.H. Zinder, and J.M. Gossett. 1995. "Comparison of Alternative Electron Donors to Sustain PCE Anaerobic Reductive Dechlorination." In R.E. Hincee and A. Leeson, (Eds.): Bioremediation of Chlorinated Solvents, *Third Bioreclamation Symposium 4*: 9-16. Battelle Press, Columbus, OH..
- Fetter, C.W. 1994. Applied Hydrology. 3rd Edition. Merrill Publishing Company, Columbus, OH.
- Freedman, D.L. and J.M. Gossett. 1991. "Biodegradation of Dichloromethane in a Fixed-Film Reactor Under Methanogenic Conditions." pp. 113-133 in Hincee, R.E., and R.F. Olfenbuttel(eds.), On-Site Bioreclamation Processes for Xenobiotic and Hydrocarbon Treatment, Boston, Butterworth-Heinemann Publishers (1991).
- Freedman, D.L. and J.M. Gossett. 1989. "Biological Reductive Dechlorination of Tetrachloroethylene and Trichloroethylene to Ethylene under Methanogenic Conditions." *Applied and Environmental Microbiology* 55(9): 2144-2153.
- Freedman, D.L. and J.M. Gossett. 1991. "Biodegradation of Dichloromethane and Its Utilization as a Growth Substrate under Methanogenic Conditions." *Applied and Environmental Microbiology* 57(10): 2847-2857.
- Freeze R.A., and J.A. Cherry. 1979. Groundwater. Prentice-Hall, Englewood Cliffs, NJ.
- Gerhardt, P., R. Murry, W. Wood, and N. Krieg (eds). 1994. *Methods for General and Molecular Bacteriology*, American Society for Microbiology, Washington, D.C.
- Gibson, S.A. and G.W. Sewell. 1992. "Stimulation of Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by Addition of Short-Chain Organic Acids or Alcohols." *Applied and Environmental Microbiology* 58(4): 1392-1393.
- Gibson, S.A., D.S. Roberson, H.H. Russell, and G.W. Sewell. 1994. "Effects of Three Concentrations of Mixed Fatty Acids on Dechlorination of Tetrachloroethene in Aquifer Microcosms." *Environmental Toxicology and Chemistry* 13(3): 453-460.
- Gossett, J.M. 1987. "Measurement of Henry's Law constants for C₁ and C₂ chlorinated hydrocarbons". *Environmental Science and Technology* 21(12):202-208.
- Gossett, J.M., T.D. DiStefano, and M.A. Stover. 1994. *Biological Degradation of Tetrachloroethylene in Methanogenic Conditions*. U.S. Air Force Technical Report No. AL/EQ-TR-1983-0026, USAF Armstrong Laboratory, Environics Directorate, Tyndall AFB, FL.
- Hach Co. 1990. Hach Company Catalog: Products for Analysis. Ames, IA.

- Heath, R.C. 1983. Basic Ground-Water Hydrology. U.S. Geological Survey Water-Supply Paper 2220.
- Holliger, C., G. Schraa, A.J.M. Stams, and A.J.B. Zehnder. 1993. "A Highly Purified Enrichment Culture Couples the Reductive Dechlorination of Tetrachloroethene to Growth." *Applied and Environmental Microbiology* 59(9): 2991-2997.
- Hopkins, G.D., L. Semprini, and P.L. McCarty. 1993. "Microcosm and In Situ Field Studies of Enhanced Biotransformation of Trichloroethylene by Phenol-Utilizing Organisms." *Appl. Environ. Microbiol.* 59: 2277-2285.
- Hvorslev, M.J. 1951. "Time Lag and Soil Permeability in Groundwater Observations." *U.S. Army Corps Engrs. Waterways Exp. Sta. Bull.* 36, Vicksburg, MS.
- Johnson Division. 1975. *Ground Water and Wells*. Saint Paul, MN.
- Jones, R.E. and D.M. Byrd III. 1989. "Recent Scientific Developments that Affect the Assessment of Risk Posed by Trichloroethylene and Perchloroethylene." *Journal of Testing and Evaluation* 17(2): 90-94.
- Kampbell, D.H., J.T. Wilson, and S.A. Vandegrift. 1989. Dissolved Oxygen and Methane in Water by a GC Headspace Equilibrium Technique. *Intern. J. Environ. Anal. Chem.*, 36: 249-257.
- Kleopfer, R.D., D.M. Easley, B.B. Haas, Jr., T.G. Deihl, D.E. Jackson, and C.J. Wurrey. 1985. "Anaerobic Degradation of Trichloroethylene in Soil." *Environmental Science and Technology* 19(3): 277-280.
- Levenspiel, O. 1972. *Chemical Reaction Engineering*. John Wiley & Sons. New York, NY.
- Lovley, D.R., F.H. Chapelle, and J.C. Woodward. 1994. "Use of Dissolved H₂ Concentrations to Determine Distribution of Microbially Catalyzed Redox Reactions in Anoxic Groundwater." *Environmental Science and Technology* 28(7): 1205-1210.
- Mackay, D., and W.Y. Shiu. 1981. "A Critical Review of Henry's Law Constants for Chemicals of Environmental Interest." *J. Phys. Chem. Ref. Data* 10(4): 1175-1199.
- Major, D.W., E.W. Hodgins, and B.J. Butler. 1995. *Field and Laboratory Evidence of In Situ Biotransformation of Tetrachloroethene to Ethene and Ethane at a Chemical Transfer Facility in North Toronto*. Guelph-Ontario, Canada.
- Maymó-Gatell, X., V. Tandoi, J.M. Gossett, and S.H. Zinder. 1995. "Characterization of an H₂-Utilizing Enrichment Culture That Reductively Dechlorinates Tetrachloroethene to Vinyl Chloride and Ethene in the Absence of Methanogenesis and Acetogenesis." *Applied and Environmental Microbiology* 61(11): 3928-3933.
- Montgomery J.H., and L. Welkom. 1990. Groundwater Chemicals Desk Reference. Lewis Publishers, Inc. Chelsea, MI.
- Nelson, M.J.K, S. Montgomery, and P. Prichard. 1988. "Trichloroethylene Metabolism by Microorganisms that Degrade Aromatic Compounds." *Applied and Environmental Microbiology*. 54(2): 604-606.

- Parsons, F., and G.B. Lage. 1985. "Chlorinated Organics in Simulated Groundwater Environments." *In: Research and Technology*. pp. 52-59.
- Parsons, F., P.R. Wood, and J. DeMarco. 1984. "Transformations of Tetrachloroethene and Trichloroethene in Microcosms and Groundwater." *In Research and Technology*, pp. 56-59.
- Puls, R.W., and M.J. Barcelona. 1996. *Low-Flow(Minimal Drawdown) Ground-Water Sampling Procedures*. EPA/540/S-95/504. U.S. Environmental Protection Agency.
- Roberts, A.L., and P.M. Gschwend. 1994. "Interaction of Abiotic and Microbial Processes in Hexachloroethane Reduction in Groundwater." *Journal of Contaminant Hydrology* 16: 157-174.
- Roberts, P.V., G.D. Hopkins, D.M. Mackay, and L. Semprini. 1990. "A Field Evaluation of In-Situ Biodegradation of Chloroethenes: Part 1, Methodology and Field Site Characterization." *Ground Water* 28(4): 591-604.
- Roberts P.V., L. Semprini, G. Hopkins, D. Grbic-Galic, P. McCarty, and M. Reinhard. 1989. *In-Situ Aquifer Restoration of Chlorinated Aliphatics by Methanotrophic Bacteria*. EPA/600/S2-89/033. U.S. Environmental Protection Agency.
- Russell H.H., J. Matthews, and G. Sewell. 1992. *TCE Removal from Contaminated Soil and Ground Water*. EPA/540/S-92/002. U.S. Environmental Protection Agency.
- Sayles, G.D., P. Mihopoulos, and M. Suidan. 1997. "Anaerobic Bioventing of PCE." *Fourth International In Situ and On-Site Bioremediation Symposium 1*: 353-359. New Orleans, LA.
- Schroth, M.H., J. Istok, M. Hyman, and K. O'Reilly. 1997. "Field-Scale Measurements of In Situ Microbial Metabolic Activities." *Fourth International In Situ and On-Site Bioremediation Symposium 2*: 387-392. New Orleans, LA.
- Semprini, L., P.V. Roberts, G.D. Hopkins, and P.L. McCarty. 1990. "A Field Evaluation of In-Situ Biodegradation of Chloroethenes: Part 2, Results of Biostimulation and Biotransformation Experiments." *Ground Water* 28(5): 715-727.
- Sewell, G.W. and S.A. Gibson. 1991. "Stimulation of the Reductive Dechlorination of Tetrachloroethene in Anaerobic Aquifer Microcosms by the Addition of Toluene." *Environmental Science and Technology* 25(5): 982-984.
- Sewell, G.W., and S.A. Gibson. 1996. *Microbial Ecology of Adaption and Response in the Subsurface*. Symposium on Natural Attenuation of Chlorinated Organics in Ground Water. EPA/540/R96/509. U.S. Environmental Protection Agency.
- Shati, M.R., D. Ronen, and R. Mandelbaum. 1996. "Method for in Situ Study of Bacterial Activity in Aquifers." *Environmental Science and Technology* 30(3): 2646-2653.
- Sims, J.L., J.M. Suflita, and H.H. Russell. 1992. *In-Situ Bioremediation of Contaminated Ground Water*. EPA/540/S-92/003. U.S. Environmental Protection Agency.
- Skeen, R.S., J. Gao, and B.S. Hooker. 1995. "Kinetics of Chlorinated Ethylene Dechlorination Under Methanogenic Conditions." *Biotechnology and Bioengineering* 48: 659-666.

- Smatlak, C.R., J.M. Gossett, and S.H. Zinder. 1996. "Comparative Kinetics of Hydrogen Utilization for Reductive Dechlorination of Tetrachloroethene and Methanogenesis in an Anaerobic Enrichment Culture." *Environmental Science and Technology* 30(9): 2850-2858.
- Staudinger, J., and P. Roberts. 1996. "A Critical Review of Henry's Law Constants for Environmental Applications". *Critical Reviews in Environmental Science and Technology*. 26(3): 205-297.
- Stecher, P.G., M. Windholz, D.S. Leahy, D.M. Bolton, and L.G. Eaton. (Eds.) 1968. The Merck Index. 8th. ed. Merck & Co., Inc., Rahway, NJ.
- Suflita, J.M., and G.W. Sewell. 1991. *Anaerobic Biotransformation of Contaminants in the Subsurface*. EPA/600/M-90/024. U.S. Environmental Protection Agency.
- Tandoi, V., T.D. DiStefano, P.A. Bowser, J.M. Gossett, and S.H. Zinder. 1994. "Reductive Dechlorination of Chloroethenes and Halogenated Ethanes by a High-Rate Anaerobic Enrichment Culture." *Environmental Science and Technology* 28(5): 973-979.
- U.S. Environmental Protection Agency. 1983. *Methods for Chemical Analysis of Water and Wastes*. EPA/16020-07-71. Cincinnati, OH.
- U.S. Environmental Protection Agency. 1986. *RCRA Ground-Water Monitoring Technical Enforcement Guidance Document*.
- U.S. Environmental Protection Agency. 1989. *Guide for Conducting Treatability Studies Under CERCLA*. EPA/540/2-89/058.
- Vroblesky, D.A. and F.H. Chapelle. 1994. "Temporal and spatial changes of terminal electron-accepting processes in a petroleum hydrocarbon-contaminated aquifer and the significance for contaminant biodegradation." *Water Resources Research* 30(5): 1561-1570.
- Wiedemeier, T.H., M. Swanson, and M. Pound. 1996. Guidelines for Evaluating Remediation by Natural Attenuation of Chlorinated Solvents in Groundwater. Rev. 0. San Antonio, TX: U.S. Air Force Center for Environmental Excellence. In draft.
- Young R.G., and J. Gossett. 1997. "Effect of Environmental Parameters and Concentrations on Dechlorination of Chloroethenes. In: *In Situ and On-Site Bioremediation* 3: 61.
- Zehnder, A.J.B., and K. Wuhrmann. 1976. "Titanium(III) Citrate as a Nontoxic Oxidation-Reduction Buffering System for the Culture of Obligate Anaerobes". *Science* 194:1165-1166.
- Zinder, S.H., and J.M. Gossett. 1995. "Reductive Dechlorination of Tetrachloroethene by a High Rate Anaerobic Microbial Consortium." *Environmental Health Perspectives* 103: 5-7.